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AI Models Detecting and Classifying Brain Tumors, Can They Be Trusted? By Devin Lee

Abstract

Brain tumors are a severe neurological condition that can affect essential brain functions such as vision, movement, speech, and memory. Detecting a brain tumor early is pivotal, as earlier detection provides more treatment options and increases the chances of successful recovery. Magnetic Resonance Imaging (MRI) is commonly used to create detailed images of brain tumors, but reviewing large numbers of scans is time-consuming for radiologists. This research evaluates machine learning models like Convolutional Neural Networks (CNNs), Decision Trees, Random Forests, and K-Nearest Neighbors (KNN to classify MRI scans into Gliomas, Pituitary, Meningioma, and noTumor categories. All images were resized to 64 by 64 pixels and split into training, testing, and validation sets. Due to an imbalanced dataset with fewer images without tumors, the noTumor class was weighted. A majority vote ensemble was used to combine the models. F1 score was identified as the key metric for performance evaluation on this imbalanced dataset.

Introduction

Brain Tumors are a severe neurological condition that can affect essential brain functions such as vision, movement, speech, and memory. Thus, detecting a brain tumor early is pivotal. As the earlier you can detect it, the more treatment options are available, therefore increasing the chances of successful recovery. Magnetic Resonance Imagery (MRI) is one of the most commonly used imaging techniques for detecting brain tumors due to it creating detailed images. However, interpreting MRI scans requires experienced radiologists, and reviewing large numbers of images can be time-consuming. As the demand for medical imaging continues to grow, researchers have begun exploring artificial intelligence (AI) as a tool to assist healthcare professionals by analyzing MRI scans quickly and accurately.

Brain Tumors occur when there's a growth of cells in or near the brain. There are different types of brain tumors, some that grow quickly and some that grow slowly. Typically, doctors classify brain tumors by which part of the brain the growth began from. The brain tumors that my AI model is examining are Gliomas, Pituitary, and Meningioma tumors.

It is so crucial for this research to be implemented, as in this research,(Identifying the best machine learning algorithms for brain tumor segmentation, progression assessment, and overall survival prediction in the BRATS Challenge.) the researchers explain on how brain tumors are dangerous, especially gliomas. They talk about how machine learning models could help predict the probability of a patient surviving. In addition, some gliomas are inoperable, but surgeons still attempt to work on it, leading to the patient possibly dying. By using machine learning, it can predict the probability of the patient surviving if a resection is attempted on the tumor. This allows doctors to recognize the type of tumor and improve patient survivability. Another researcher had a similar idea, improving patient survivability. Ali Isin (Review of MRI-based brain tumor image segmentation using deep learning methods. Procedia Computer

Science) stated that manually segmenting MRI brain images are time-consuming, due to the large number of MRI scans generated in hospitals. Making this process automatic will thus save much more time and allow diagnosis to arrive quicker. This allows patients more time to get treatment and potentially increase their chances for survival.

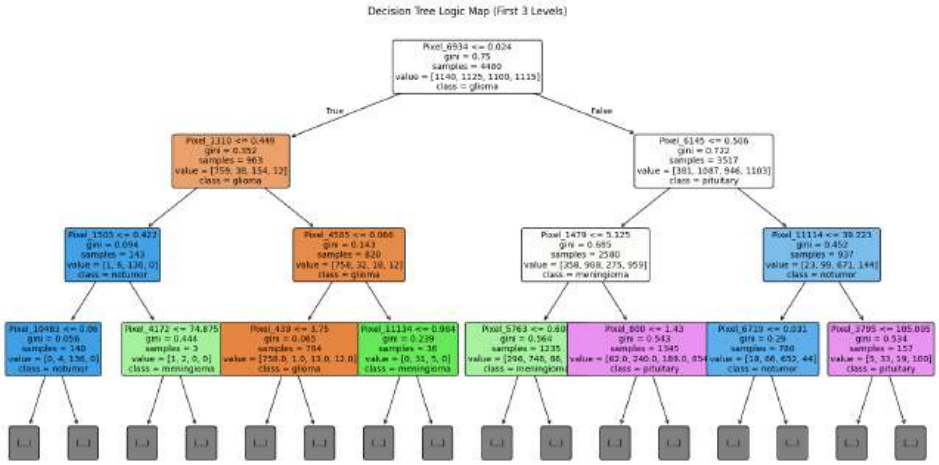
Recent developments in machine learning created many image detection models that could be implemented to scan an MRI Brain Scan. Some models are Convolutional Neural Networks (CNNs), Decision Trees, Random Forests, and K-Nearest Neighbors (KNN). This research investigates which AI model provides the highest accuracy for detecting and classifying brain tumors from MRI Scans by comparing the performance of the machine learning models stated above through evaluation metrics such as accuracy, precision, recall, confusion matrixes, and plot diagrams. The results of this study will help others understand the strength and limitations of each model and contribute to the growing field of AI-assisted medical diagnosis.

Materials and Methods

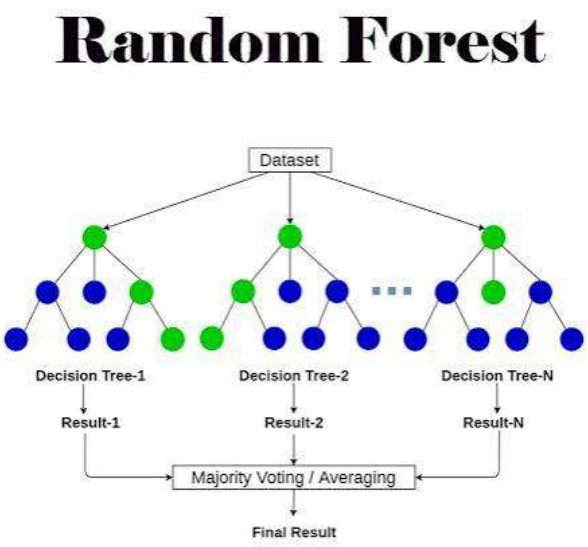
The dataset used in this study is the Brain Tumor MRI Dataset from Kaggle (Nickparvar, 2024), which includes data derived from Figshare, Br35H, and BRATS datasets. Link to Dataset Preprocessing involved making all the images 64 by 64 pixels, although by making it 64 by 64 pixels it removes small details, it was done to improve computing efficiency. There was a problem with the dataset, as the noTumor images had significantly fewer images than the images with brain tumors. I fixed this issue by weighting the noTumor dataset by 3.

One important step in the AI model training process is splitting the dataset into training, testing, and validation. It is important as it allows researchers to see if an AI model performs well on not only the data they have seen, but also on new, unseen data. If a model is trained on the same images, it will lead to overfitting, where it just memorizes the dataset. With a testing dataset in the mix, the AI model has to learn patterns in order to pass the "test." This provides a more accurate measure of how the AI model would react to an unpredictable real world scenario. The training set (20%) tests the model to learn patterns in the dataset. The Validation set (20%) tests the model on new never before seen data. It checks what hyperparameters the model messes up on, and then changes them. This helps the model improve. The Testing set (60%) is where the test arrives. All of the data within are data the model has not seen before. Here we can see how the model performs after all of the tuning.

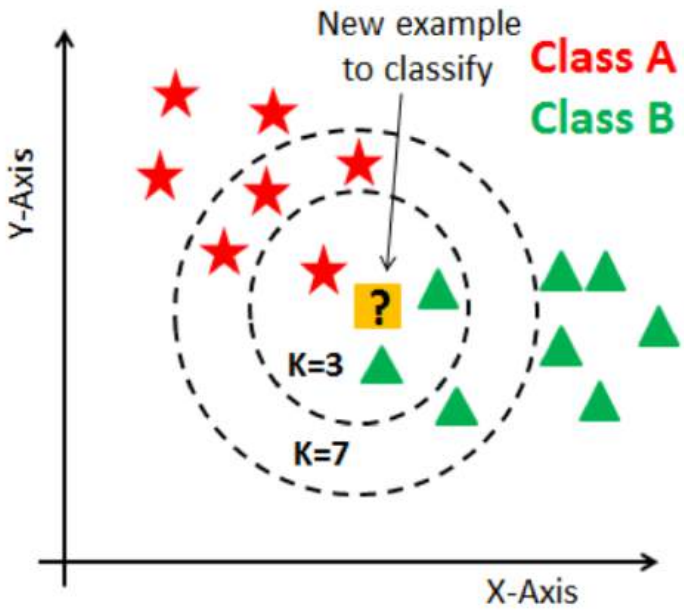
Decision Tree: A Decision Tree is a supervised machine learning algorithm that classifies data by repeatedly splitting it into smaller groups based on feature values. The model creates a tree-like structure where choices branch out from the center. For brain MRI classification, each image is converted into numerical features, and the Decision Tree learns a sequence of rules that distinguishes between glioma, meningioma, pituitary tumors, and healthy brain scans. Decision Trees are easy to interpret and visualize, but they are prone to overfitting because a single tree may memorize patterns present only in the training data.



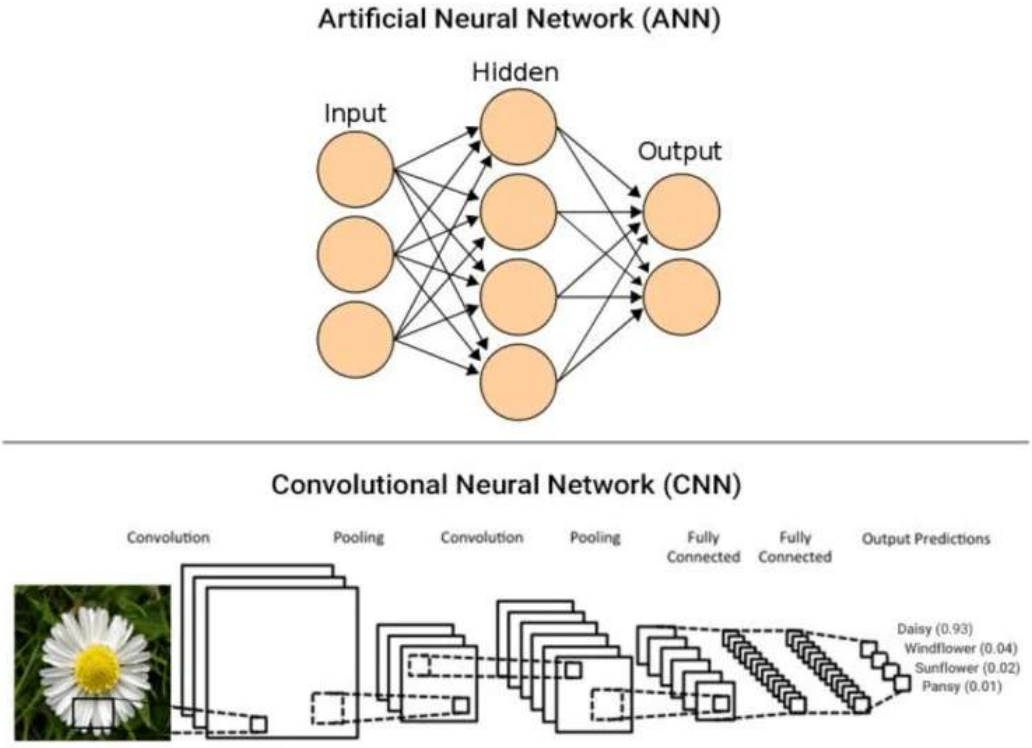
Random Forest: A Random Forest is an ensemble learning algorithm that combines many Decision Trees to improve classification performance and reduce overfitting. Each tree is trained on a random subset of the training data and a random subset of features. During prediction, every tree votes for a class, and the majority vote becomes the final prediction. By averaging the outputs of many trees, Random Forests produce more stable and accurate predictions than a single Decision Tree.



K-Nearest Neighbors (KNN): K-Nearest Neighbors (KNN) classifies a sample according to the labels of its nearest neighboring examples in the training set. The algorithm calculates distances between the input sample and all training samples using metrics such as Euclidean distance. The class most frequently represented among the k closest neighbors becomes the predicted class. KNN does not construct an explicit mathematical model during training. Instead, it stores the training data and performs computations during prediction.



Convolutional Neural Networks (CNN): A Convolutional Neural Network (CNN) is a deep learning architecture specifically designed for image analysis. CNNs automatically learn hierarchical features directly from raw image pixels without requiring manual feature extraction. Simply put, it finds patterns in images like texture, shapes, and sizes. Early layers learn simple features such as edges, while deeper layers recognize more sophisticated structures unique to each tumor category.



Model Combining (Majority Vote)

Having each model separately presented problems with performance and individual limitations. We combined the models using a majority vote of all the machine learning models to see how they perform together.

Metrics

The performance of the models is evaluated using accuracy, precision, recall, F1 score, confusion matrixes, and performance plots (learning curves).

Precision Issue: If you predict that one person has cancer, and they do, the precision for that will be 100%.

$$\text{Precision} = \frac{True\ Positive}{True\ Positive + False\ Positive}$$

Recall Issue: If you predict everyone has cancer, the recall will be 100%

$$\text{Recall} = \frac{True\ Positive}{True\ Positive + False\ Negative}$$

F1 Score: For this specific dataset, the F1 score is the appropriate metric to determine accuracy for the AI model. In reality, the F1 score works better with an imbalanced dataset, where it focuses on a specific topic to improve the accuracy in it.

$$The\ F_1\ Score\ is\ given\ by:$$

$$F_1 = 2 * \frac{Precision * Recall}{Precision + Recall}$$

Confusion Matrix: A confusion matrix is a table that shows how well an AI model makes predictions by comparing its predictions to the actual results. Instead of only showing the overall accuracy, it shows the number of correct and incorrect predictions (true positives, true negatives, false positives, and false negatives). In brain tumor detection, a false negative is especially serious because it means a tumor was not detected. It helps researchers see which data types the AI model struggles with the most.

Performance Plot (Learning Curve): A performance plot, or learning curve, is a graph that shows how an AI model improves during training by displaying accuracy or loss over time for both training and validation datasets. This helps researchers decide when the model has been trained enough and shows whether overfitting is occurring.

There are many issues with models, especially overfitting.

Overfitting occurs when the model memorizes the training dataset instead of important features that identify an object. Instead of memorizing meaningful characteristics such as size, shape, and texture of a tumor, it memorizes less important characteristics such as the exact position or patterns only in the training dataset. As a result, the model performs well on training images but fails on testing datasets. One reason you know overfitting is happening is if your accuracy on the training dataset is more than your testing dataset.

Overfitting can also occur due to distribution shifts. Distribution shifts occur when real world data is much more different from the data within the dataset. For example, if tumors in the dataset are all centered in the middle of the image, the model will begin to only look at the center of the screen, ignoring the corner pixels. Resizing images can also cause issues. Just because a model is good in one dataset doesn't mean it's good in every dataset.

Researchers have some methods to fix overfitting and distribution shifts. Some ways are Data Augmentation and dropout.

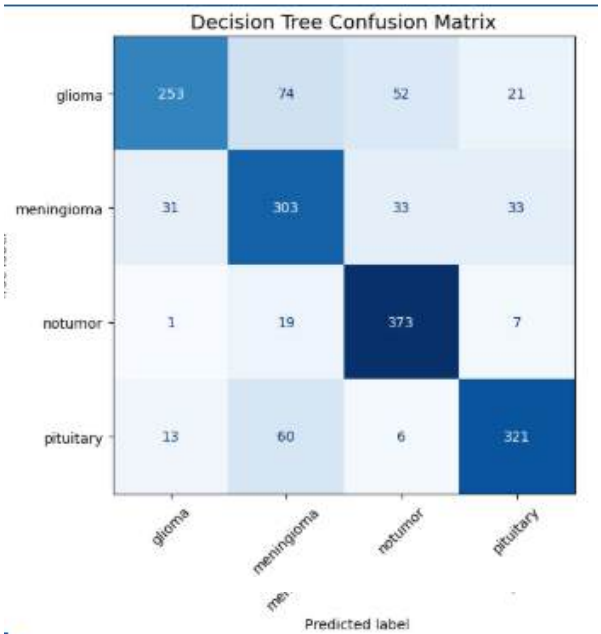
Data Augmentation: Creating patterns from your dataset that you would see in the real world (like picking more diverse data) so the model learns general patterns rather than generic training set patterns.

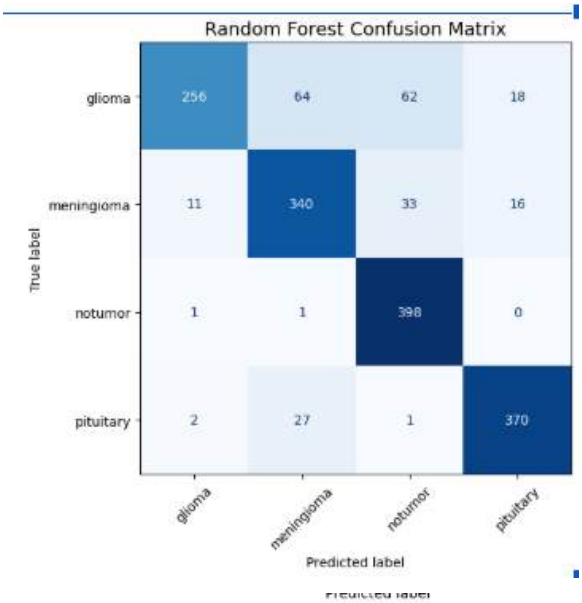
Dropout: During training, dropout randomly turns off a percentage of neurons in the neural network (or connections). For example, a dropout of 10% randomly removes pixels or connections, forcing the model to look at other parts of the image to learn other patterns. If the dropout rate is too low, the model may still overfit. If it is too high (like 15% or bigger), the model may not have enough information to learn effectively and may start picking randomly.

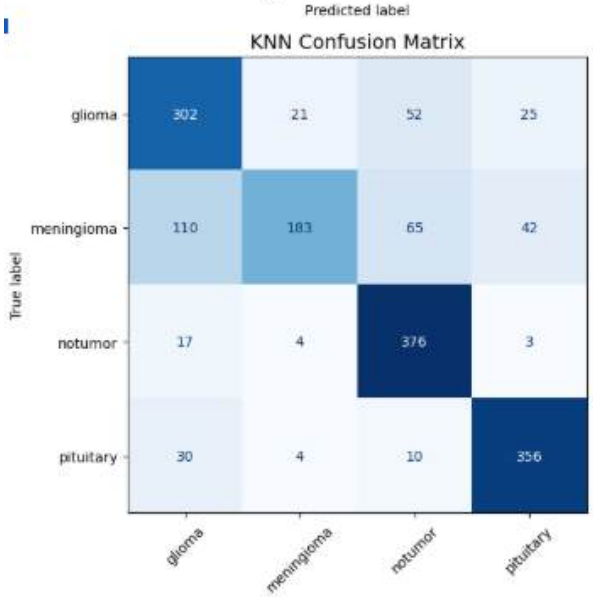
Results

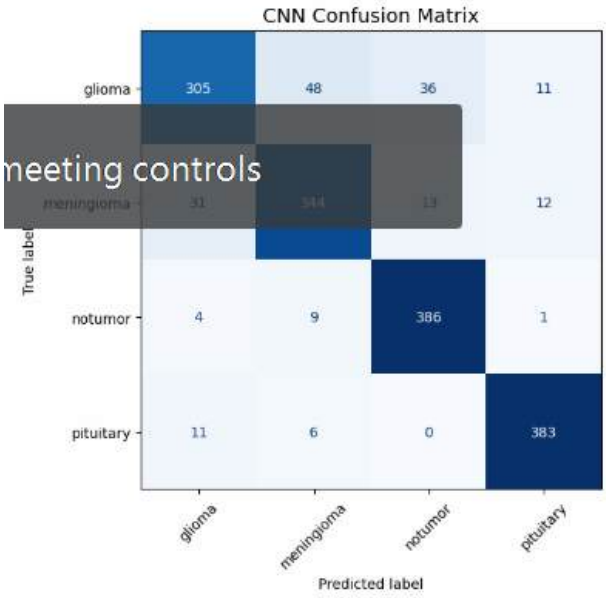
The metrics for training and testing for every model (accuracies, F1 score, precision, recall, confusion matrixes, learning curves)

Models	Training Accuracy	Testing Accuracy	Precision	Recall	F1 Score
Decision Tree	0.98	0.78	0.79	0.78	0.78
Random Forest	1.00	0.85	0.86	0.85	0.85
K Nearest Neighbors (KNN)	0.90	0.76	0.78	0.76	0.76
Convolutional Neural Network (CNN)	0.98	0.89	0.89	0.89	0.89
Ensemble	N/A	0.86	0.86	0.86	0.86









Discussion

In this research paper, multiple machine learning models (CNNs, Decision Trees, Random Forests, and KNN) were developed and compared to detect and classify brain tumors from MRI scans. The dataset was split into training, validation (20%), and testing (60%) sets, the images were resized to 64x64 pixels, and the noTumor class was weighted to fix dataset imbalance.The models were evaluated using precision, recall, accuracy, F1 score, confusion matrices, and learning curves, identifying F1 score as the best universal metric. I also analyzed problems with AI like overfitting and distribution shifts, and explained how dropouts and data augmentation are used to fix them. Finally, I combined all models using a majority vote to evaluate their combined performance. In the end, we learned that the best machine learning

model for image detection was the CNN, which generally had an average of 89% accuracy in the dataset.

In the future, I hope that I could incorporate dropouts in my models. I also hope that I could add more to the models, like making it more accurate. In addition, if I could have the model be tested on real world data from real doctors, it would improve the learning process. I also hope the model could be expanded to classifying the grade of a tumor, not simply the type. Lastly, I hoped that the images could be preprocessed to a higher scale, such as 256 by 256 pixels, allowing more detail to be seen. This allows the machine learning models to see smaller patterns, improving accuracy.

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Academic Stress and Eating Changes Across the School Years: A Cross-Sectional Survey of 1,000 Students Aged 10 to 18 By Laasya Kolli

Abstract

Academic stress is one of the most common sources of distress reported by school students, and stress is known to alter appetite, meal patterns and body-related thoughts. Most studies of stress and eating in young people have focused on older adolescents or university students, and few have followed the same set of questions across the whole span from primary to senior secondary school. This study surveyed 1,000 school students aged 10 to 18 years (grades 2 to 12) in Andhra Pradesh, India, using an anonymous Google Forms questionnaire with four yes/no items on academic stress and four on stress-related eating. Item endorsement was summarised with 95% confidence intervals, age trends were tested with the Cochran-Armitage test and Cramer's V, and associations between stress and eating were estimated with crude odds ratios, Holm-corrected item-by-item tests, multivariable logistic regression adjusted for age, Poisson and ordinal models for the count of eating changes, and an age-partialled correlation. Pressure to achieve high grades was reported by 69.0% of students, and the proportion reporting high academic stress (three or four of four items) rose from 7.3% at age 10 to 75.3% at age 18 (Cramer's V for age band = 0.43, $p < 0.001$). Stress-related eating changes were reported by 22.8% to 35.9% of students depending on the item, with only a shallow age gradient. Skipping meals when stressed was modestly more common among high-stress students (27.7% versus 20.7%; age-adjusted odds ratio 1.39, 95% CI 1.00 to 1.93), a suggestive rather than conclusive association, but no other eating outcome was associated with stress after adjustment, no single stress item predicted any eating outcome in multivariable models, and the age-partialled correlation between the stress and eating counts was 0.00. The study had 78% to 88% power to detect an odds ratio of 1.5, and results were unchanged in the 876 students whose age and grade were in the expected relation to each other. Academic stress therefore appears to rise sharply across the school years while reported eating changes remain at a roughly constant background level, and there was little evidence of a meaningful link between the two after age adjustment, with meal skipping the one behaviour showing a modest association. These findings argue for stress-reduction efforts that start well before the final examination years and for protecting regular meals during periods of high academic pressure.

Introduction

Every year, millions of school students sit examinations that they, their families and their teachers treat as decisive for their futures. In India and much of South Asia, the pressure surrounding these examinations is unusually intense: middle-class students spend about a third of their waking hours on schoolwork and report their most negative emotional states while doing it (Verma et al.), and surveys of Indian secondary school students have repeatedly found high rates of academic stress together with depressive symptoms (Deb et al.; Jayanthi et al.). Academic stress in adolescence is not a trivial concern. Adolescence is a sensitive period in which habits of

coping, sleeping and eating are laid down that persist into adult life (Dahl et al.), and mental health problems in young people are now recognised as a global public health priority (Patel et al.).

One of the ways stress reaches the body is through eating. Stress hormones act on appetite in two directions: the acute response, driven by the sympathetic nervous system and corticotropin-releasing hormone, tends to suppress hunger, whereas prolonged or repeated stress raises cortisol and increases the drive for energy-dense, highly palatable food (Adam and Epel; Dallman). In adults, these mechanisms translate into a well-documented pattern in which roughly equal proportions of people eat more and eat less under stress, with the direction depending on individual traits such as dietary restraint (Oliver et al.; Torres and Nowson). Stress-related eating is also linked to longer-term risks, including obesity (Sinha and Jastreboff), and in adolescence negative affect, dieting and body dissatisfaction are established risk factors for the onset of eating disorders (Stice et al.).

The evidence in children and adolescents is thinner and more mixed. A systematic review and meta-analysis found that stress in youth was associated with both increased and decreased intake, with emotional eating the most consistent finding (Hill et al.). A large cross-sectional study of primary-school children linked stress to more emotional eating and poorer dietary patterns (Michels et al.), an experimental study showed stress-induced snacking in children who habitually restrain their eating (Roemmich et al.), and among Korean high school students, academic stress was associated with greater consumption of sweet foods (Kim et al.). What is missing from this literature is a single sample that spans the full age range of schooling, uses the same questions throughout, and asks directly about academic rather than general stress.
This study was designed to fill that gap. Using a brief anonymous survey completed by 1,000 students aged 10 to 18 years, it addresses three questions. First, how commonly do school students report academic stress and stress-related changes in eating, and how do these reports change across age? Second, are individual stress indicators, or the number of indicators a student endorses, associated with each eating behaviour once age is taken into account? Third, is there a recognisable group of students in whom high academic stress and multiple eating changes co-occur, and does that group grow with age? The hypothesis at the outset was that both stress and stress-related eating would rise with age and that students reporting more stress would report more eating changes.

Background and Literature Review

Academic stress in the school years

Stress, in the influential formulation of Lazarus and Folkman, arises when a person judges the demands of a situation to exceed the resources available to meet them. For school students the demands are workload, evaluation and the expectations of parents and teachers; the resources are time, ability, support and coping skills. Pascoe, Hetrick and Parker reviewed the evidence on stress in secondary and higher education and concluded that academic stress is

associated with poorer sleep, reduced physical activity, worse diet and lower wellbeing, and that these effects are visible in adolescents and not only in university students. In India, Deb, Strodl and Sun found that a majority of urban higher secondary students reported academic stress and that parental pressure was a leading contributor, while Jayanthi, Thirunavukarasu and Rajkumar reported that adolescents with academic stress were more than twice as likely to have depressive symptoms. Reddy, Menon and Thattil, studying university students in India, identified examinations and workload as the dominant sources of stress. Across these studies, stress is measured mostly in older adolescents; the trajectory of academic stress from late childhood upward is far less well described.

How stress changes eating

The physiological account of stress and eating is now reasonably settled. Adam and Epel describe how glucocorticoids released under chronic stress increase the salience of palatable food and interact with the brain reward system, producing "comfort eating" that is reinforced because it dampens the stress response. Dallman extends this to explain stress-induced obesity. Torres and Nowson, reviewing human studies, note that the same stress exposure can increase or decrease intake depending on the person, and Oliver, Wardle and Gibson demonstrated experimentally that stressed emotional eaters ate more sweet, high-fat food while others ate less. These findings matter for measurement: a survey item asking whether stress "changes how much I eat" will be endorsed by both hyperphagic and hypophagic responders, and its relationship with stress will be a blend of two opposite effects.

Stress and eating in young people

Hill and colleagues pooled studies in children and adolescents and found small but consistent associations between stress and unhealthy eating, with emotional eating the clearest. Michels and colleagues, in a cross-sectional study of 437 Belgian children aged 5 to 12, found that stress was associated with emotional eating and consumption of sweet foods. Roemmich, Wright and Epstein showed that children of about nine years who were high in dietary restraint snacked more after a laboratory stressor. Kim and colleagues linked academic stress to sweet food intake in Korean students facing an examination-driven system with similarities to India. Neumark-Sztainer and colleagues, in a ten-year cohort, showed that dieting and irregular eating in adolescence predict disordered eating in young adulthood, and Stice, Marti and Durant identified body dissatisfaction, dieting and negative affect as risk factors for the onset of eating disorders. Together these studies establish that stress-related eating in youth is real, measurable and consequential, while leaving open how it relates to academic stress specifically and how early it appears.

Materials and Methods

Study design and setting

This was a cross-sectional, questionnaire-based survey of school students. Data were collected through an anonymous online form (Google Forms) distributed to school students in grades 2 to 12 across the state of Andhra Pradesh, India, between May and August 2026. To protect respondent privacy, the survey was fully anonymous: the participating schools were not recorded, and no school, teacher or student is identified in this report. The form was shared electronically and completed by students on their own devices outside supervised class time. The study was designed and reported with reference to the STROBE recommendations for cross-sectional studies (von Elm et al.).

Participants

Students aged 10 to 18 years enrolled in grades 2 to 12 were eligible. A convenience sampling approach was used, with the questionnaire circulated to students across multiple schools in the state. Because distribution was open and anonymous, the total number of students who received the link was not recorded, so a formal response rate cannot be calculated; this is acknowledged as a limitation. Forms with any missing answer were excluded before analysis, and the analysed dataset contained 1,000 complete responses.

Questionnaire

The questionnaire was written for this study in English and was not formally piloted before use. It recorded age in years and current grade, followed by eight closed yes/no items. Four items addressed academic stress: (Q3) "I feel overwhelmed by my academic workload"; (Q4) "I frequently worry about my academic performance"; (Q5) "I feel pressure to achieve high grades"; and (Q6) "Academic stress negatively affects my daily life". Four addressed eating: (Q7) "I skip meals when I am academically stressed"; (Q8) "Stress changes how much I eat"; (Q9) "I think more about my body weight or appearance when I am stressed"; and (Q10) "Academic stress has influenced my eating habits". Items were numbered Q3 to Q10 because Q1 and Q2 were the age and grade fields. No item recorded sex, height, weight or socioeconomic background. A yes/no format was chosen so that the youngest respondents could complete the form without help; the limitations this imposes are discussed later.

Derived variables

"Yes" was coded 1 and "No" 0. A stress count (0 to 4) was formed by adding the four stress items, and students endorsing three or four items were classified as reporting high academic stress. An eating count (0 to 4) was formed in the same way; two further binary outcomes were defined as endorsing at least one eating item ("any eating change") and endorsing two or more ("multiple eating changes"). Age was analysed as a continuous variable and in three bands (10 to 12, 13 to 15 and 16 to 18 years). Because age and grade are strongly collinear, age

in years was used as the developmental variable throughout; grade was used only to check the internal consistency of the demographic data (see Statistical analysis).

Ethical considerations

This study did not undergo formal institutional review, as it was an anonymous, low-risk survey carried out independently by a student researcher without institutional affiliation. Participation was voluntary, and students could decline or stop at any point without consequence. The introduction to the form explained the purpose of the survey, stated that responses were anonymous and would be used only for research, and included a parental consent statement: students were told that they should complete the survey only with the knowledge and agreement of a parent or guardian, and by proceeding they confirmed that this condition had been met. Because the survey was anonymous, this consent was self-reported and could not be independently verified. The form collected no names, contact details or other identifying information, and responses cannot be linked to individual students. No incentive was offered for participation. Because the dataset is fully anonymous, the schools and students involved cannot be re-identified. The absence of formal ethical review, and the reliance on self-reported rather than documented parental consent, are acknowledged as limitations and discussed below.

Statistical analysis

Analyses are organised in four tiers. The primary analyses were (1) the prevalence of each stress and eating item with confidence intervals, (2) the trend in each item across age, and (3) the age-adjusted association between academic stress (as the high-stress indicator and as the stress count) and each of the six eating outcomes. Secondary analyses were the 16 item-by-item cross-tabulations, the multivariable models entering all four stress items together, and the count models for the number of eating changes. Sensitivity analyses repeated the primary models in the age-to-grade concordant subset and after excluding the youngest respondents. Exploratory and post-hoc analyses, reported for completeness and not used to draw conclusions, were the stress-by-age interaction test, the comparison of the combined high-stress and multiple-eating-change pattern with independence, and the power calculation. Conclusions rest on the primary analyses; the secondary, sensitivity and exploratory analyses are reported to show that the primary result holds under different assumptions, not to search for additional significant effects.

Item endorsement was summarised as counts and percentages with Wilson 95% confidence intervals. Internal consistency of the stress and eating item sets was assessed with the Kuder-Richardson formula 20 (KR-20), the binary-item form of Cronbach's alpha, and agreement between the two most similar eating items (Q8 and Q10) was quantified with Cohen's kappa. Differences across age bands were tested with the chi-square test for binary items and the Kruskal-Wallis test for counts, and the strength of each association with age band was expressed as Cramer's V. Linear trend across single years of age was tested with the Cochran-Armitage trend test and the Spearman rank correlation. As a data quality check, the difference between

each student's reported age and grade was examined; in the Indian system children usually enter grade 1 at about age 6, so an age-to-grade gap of 5 to 7 years was treated as the expected range, and all main analyses were repeated in the subset of students within that range.

The relationship between stress and eating was examined in stages of increasing stringency. First, each of the four stress items was cross-tabulated against each of the four eating items, giving 16 crude odds ratios (ORs) with Fisher's exact p values; because these are multiple related tests, p values were corrected with the Holm procedure. Second, crude ORs compared students with high stress against all others for each of the six eating outcomes. Third, binary logistic regression models were fitted for each eating outcome with age in years as a covariate and either (a) high stress as a binary predictor, (b) the stress count as a continuous predictor, or (c) all four stress items entered together, the last model testing whether any specific stress indicator predicted eating independently of the others; model fit was summarised with McFadden's pseudo-R-squared and the likelihood-ratio test. Fourth, the eating count was modelled with Poisson regression using robust standard errors and, as a check, with proportional-odds ordinal logistic regression, both adjusted for age. Fifth, effect modification by age band was tested by adding a stress-by-age-band interaction and comparing models with a likelihood-ratio test. Sixth, the Spearman correlation between the stress and eating counts was calculated with a bootstrap 95% confidence interval (2,000 resamples) and then recalculated after removing the linear effect of age from both counts.

Two sensitivity analyses were carried out: models were refitted in the age-to-grade concordant subset, and again after excluding students aged 10 to 11 years, whose comprehension of the items may have been weakest. As an exploratory check, the observed prevalence of the combined "high stress plus multiple eating changes" pattern was compared with the prevalence expected if the two were independent. Post-hoc power was calculated for the comparison of high-stress with other students (n = 448 versus 552) at the observed outcome prevalences. Two-sided p values below 0.05 were treated as statistically significant. Analyses were carried out in Python 3 using pandas, SciPy and statsmodels.

Results

Sample

The 1,000 respondents ranged from 10 to 18 years (mean 14.0, standard deviation 2.5, median 14) and from grade 2 to grade 12 (Table 1). The three age bands were of similar size, and every single year of age contributed at least 81 students. There were no missing values. For 876 students (87.6%) the gap between reported age and grade was in the expected 5 to 7 year range; 43 reported a gap of 4 years and 81 a gap of 8 years. Only 41 students (4.1%) endorsed no stress item, while 251 (25.1%) endorsed no eating item.

Table 1: Characteristics of the 1,000 respondents.

Characteristic	n	%
Age band, years		
10 to 12 (mean 11.0)	319	31.9
13 to 15 (mean 14.0)	324	32.4
16 to 18 (mean 16.8)	357	35.7
Grade		
2 to 5	228	22.8
6 to 8	298	29.8
9 to 12	474	47.4
Age-to-grade gap within expected 5 to 7 years	876	87.6
Stress items endorsed (0 / 1 / 2 / 3 / 4)	41 / 176 / 335 / 278 / 170	4.1 / 17.6 / 33.5 / 27.8 / 17.0
Eating items endorsed (0 / 1 / 2 / 3 / 4)	251 / 408 / 256 / 76 / 9	25.1 / 40.8 / 25.6 / 7.6 / 0.9

Prevalence and measurement properties

Pressure to achieve high grades was the most widely reported stress indicator, endorsed by 690 students (69.0%, 95% CI 66.1 to 71.8), and more than half of respondents endorsed each of the other three: worry about performance (58.4%), feeling overwhelmed by workload (55.7%) and stress affecting daily life (52.9%). In all, 448 students (44.8%, 95% CI 41.7 to 47.9) met the definition of high academic stress. Eating changes were reported less often. Stress changing the amount eaten and academic stress having influenced eating habits were each endorsed by 359 students (35.9%, 95% CI 33.0 to 38.9); skipping meals when stressed by 238 (23.8%, 95% CI 21.3 to 26.5); and thinking more about weight or appearance when stressed by 228 (22.8%, 95% CI 20.3 to 25.5). Three-quarters (749; 74.9%) reported at least one eating change and 341 (34.1%) reported two or more.

Internal consistency was low for both item sets: KR-20 was 0.25 for the four stress items and 0.06 for the four eating items. The four eating items were essentially uncorrelated with one another (pairwise r between -0.05 and 0.04). Most strikingly, Q8 ("stress changes how much I eat") and Q10 ("academic stress has influenced my eating habits"), which ask closely related questions, agreed no better than chance (Cohen's kappa = 0.03): of the 359 students who endorsed Q8, only 135 (37.6%) also endorsed Q10, almost exactly the proportion among those who did not endorse Q8 (35.0%). These properties mean that the item counts should be read as tallies of separate indicators rather than as scales measuring a single construct, and the eating items are therefore analysed individually as well as in aggregate.

Age trends

Every stress indicator rose steeply with age (Table 2, Fig 1 and Fig 2). Feeling overwhelmed increased from 36.1% of 10 to 12-year-olds to 69.7% of 16 to 18-year-olds, worry about performance from 46.4% to 71.4%, pressure for grades from 52.0% to 84.6%, and stress affecting daily life from 32.9% to 70.0%; Cochran-Armitage trend tests across single years of age were highly significant for all four (z between 6.7 and 10.1, $p < 0.001$), and Cramer's V for the association with age band ranged from 0.21 to 0.31. The proportion with high academic stress quadrupled from 17.2% in the youngest band to 69.5% in the oldest (trend $z = 14.2$, Cramer's $V = 0.43$). The mean stress count rose in every successive year of age, from 1.4 at age 10 to 3.0 at ages 17 and 18, high stress rose from 7.3% of 10-year-olds to 75.3% of 18-year-olds, and the Spearman correlation between age and the stress count was 0.51 ($p < 0.001$). The rise was gradual and near-linear rather than concentrated at particular ages (Fig 2).

The eating items behaved differently. Stress changing the amount eaten showed a modest age trend (29.5%, 41.0% and 37.0% across the three bands; trend $z = 2.24$, $p = 0.025$; Cramer's $V = 0.10$), but skipping meals (trend $p = 0.068$), weight or appearance thoughts ($p = 0.12$) and stress having influenced eating habits ($p = 0.15$) did not differ significantly by age, and the Cramer's V values for all four eating items (0.05 to 0.10) were a fraction of those for the stress items. The mean eating count rose only from 1.06 to 1.29 across the three bands (Kruskal-Wallis $p = 0.014$), and its correlation with age was weak (Spearman rho = 0.10, $p = 0.002$).

Table 2: Endorsement of each item overall and by age band, with tests of age trend.

Item	**Overall % (95% CI)**	**10 to 12 y**	**13 to 15 y**	**16 to 18 y**	**Trend z (p)**	**Cramer's V**
Q3 Overwhelmed by workload	55.7 (52.6 to 58.8)	36.1	59.6	69.7	9.32 (< 0.001)	0.28
Q4 Worry about performance	58.4 (55.3 to 61.4)	46.4	55.9	71.4	6.69 (< 0.001)	0.21
Q5 Pressure for high grades	69.0 (66.1 to 71.8)	52.0	68.5	84.6	9.42 (< 0.001)	0.29
Q6 Stress affects daily life	52.9 (49.8 to 56.0)	32.9	53.7	70.0	10.07 (< 0.001)	0.31
High stress (3 or 4 items)	44.8 (41.7 to 47.9)	17.2	44.8	69.5	14.16 (< 0.001)	0.43
Q7 Skip meals when stressed	23.8 (21.3 to 26.5)	20.7	24.4	26.1	1.83 (0.068)	0.05
Q8 Stress changes amount eaten	35.9 (33.0 to 38.9)	29.5	41.0	37.0	2.24 (0.025)	0.10
Q9 Weight/appearance thoughts	22.8 (20.3 to 25.5)	21.0	20.4	26.6	1.57 (0.12)	0.07
Q10 Stress influenced eating habits	35.9 (33.0 to 38.9)	35.1	33.3	38.9	1.43 (0.15)	0.05
Any eating change	74.9 (72.1 to 77.5)	71.2	74.7	78.4	2.43 (0.015)	0.08
Multiple eating changes (2+)	34.1 (31.2 to 37.1)	29.5	33.3	38.9	2.42 (0.015)	0.07

Column percentages are the proportion answering "Yes" in each band (n = 319, 324 and 357). Trend z is from the Cochran-Armitage test across single years of age; Cramer's V measures the strength of association with the three-band age variable.

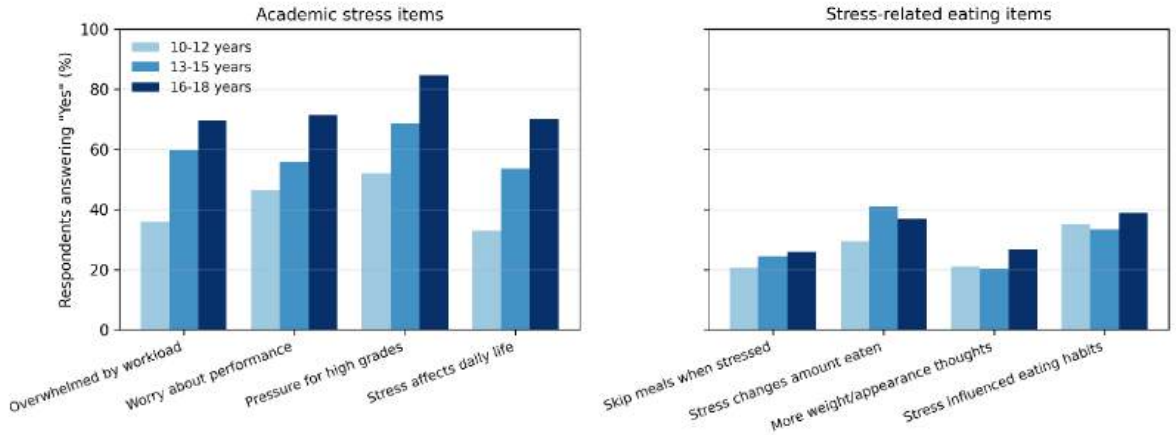


Fig 1: Proportion of students answering "Yes" to each academic stress item (left) and each stress-related eating item (right), by age band.

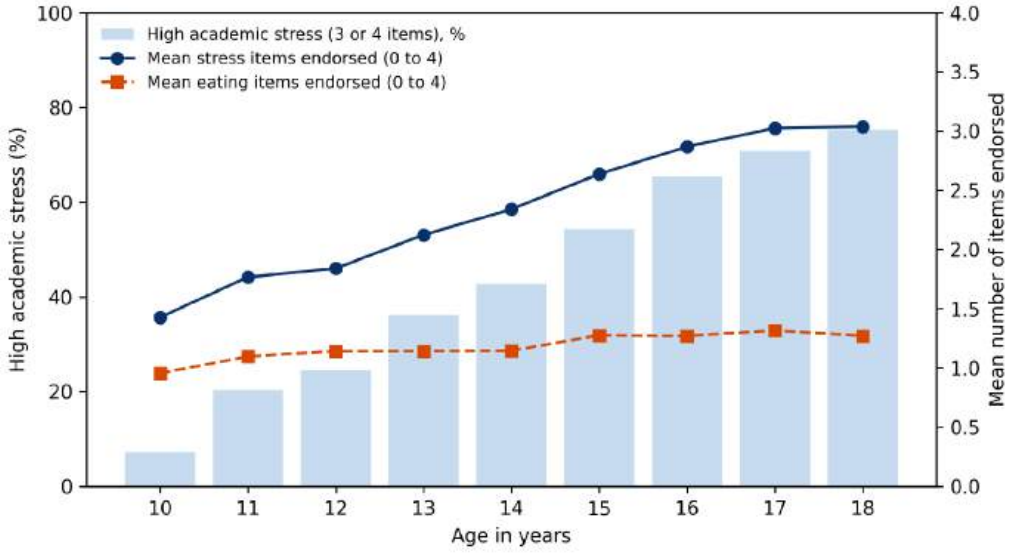


Fig 2: Year-by-year trajectory of the percentage of students with high academic stress (bars), the mean stress count (solid line) and the mean eating count (dashed line), ages 10 to 18.

Item-by-item associations between stress and eating

Cross-tabulating each stress item against each eating item produced 16 crude odds ratios, all between 0.81 and 1.33 (Table 3). Only one reached nominal significance: students who felt overwhelmed by workload were somewhat more likely to say stress changed how much they ate (OR 1.33, 95% CI 1.02 to 1.73, p = 0.034). After Holm correction for the 16 tests, however, no

association remained significant (smallest corrected p = 0.54). Worry about performance and pressure for grades were each weakly associated with meal skipping (ORs 1.32 and 1.30) but neither reached significance even before correction. Pressure for grades was, if anything, inversely related to reporting that stress had influenced eating habits (OR 0.81, 95% CI 0.61 to 1.06).

Table 3: Crude odds ratios (95% CI) for each eating item according to each stress item, with Fisher's exact p values (uncorrected; Holm-corrected).

Stress item	Q7 Skip meals	Q8 Amount eaten changes	Q9 Weight/appearance thoughts	Q10 Eating habits influenced
Q3 Overwhelmed by workload	1.13 (0.84 to 1.52); p = 0.46; 1.00	1.33 (1.02 to 1.73); p = 0.034; 0.54	1.07 (0.80 to 1.44); p = 0.70; 1.00	1.05 (0.81 to 1.37); p = 0.69; 1.00
Q4 Worry about performance	1.32 (0.98 to 1.78); p = 0.071; 1.00	1.18 (0.91 to 1.54); p = 0.23; 1.00	1.18 (0.87 to 1.59); p = 0.32; 1.00	1.06 (0.82 to 1.38); p = 0.69; 1.00
Q5 Pressure for high grades	1.30 (0.94 to 1.79); p = 0.13; 1.00	1.01 (0.76 to 1.33); p = 1.00; 1.00	0.97 (0.70 to 1.33); p = 0.87; 1.00	0.81 (0.61 to 1.06); p = 0.14; 1.00
Q6 Stress affects daily life	1.07 (0.80 to 1.43); p = 0.66; 1.00	1.13 (0.87 to 1.47); p = 0.36; 1.00	1.06 (0.79 to 1.42); p = 0.76; 1.00	1.04 (0.80 to 1.34); p = 0.79; 1.00

High stress, the stress count and eating outcomes

Students with high academic stress were more likely than others to report skipping meals when stressed (27.7% versus 20.7%; crude OR 1.47, 95% CI 1.10 to 1.97, p = 0.011). After adjustment for age the estimate fell to 1.39 (95% CI 1.00 to 1.93, p = 0.048), at the boundary of significance (Table 4 and Fig 3). For the other three eating items, for any eating change and for multiple eating changes, the crude ORs for high stress lay between 0.95 and 1.26, none was significant, and adjustment for age moved every estimate closer to 1. When the stress count was entered as a continuous predictor, each additional stress item was associated with 11% higher odds of meal skipping (adjusted OR 1.11, 95% CI 0.95 to 1.30, p = 0.18) and with no material change in the odds of any other outcome (adjusted ORs 0.93 to 1.07).

In the multivariable models entering all four stress items together, no individual stress indicator predicted any eating outcome after adjustment for the others and for age; the only coefficient to reach $p < 0.05$ was a lower odds of reporting that stress had influenced eating habits among students who felt pressure for grades (OR 0.74, 95% CI 0.55 to 0.99), and it did not survive correction for multiple testing. McFadden's pseudo-R-squared for these models was between 0.003 and 0.010, and the likelihood-ratio test against an intercept-only model was non-significant for every outcome except any eating change (p = 0.044), where the effect was carried by age rather than by any stress item. The Poisson model for the eating count gave a rate ratio of 1.02 per stress item (95% CI 0.96 to 1.07, p = 0.56) and 1.03 per year of age (p = 0.007); the ordinal logistic model gave a proportional OR of 1.01 per stress item (p = 0.83) and 1.07 per year of age (p = 0.009). In other words, age rather than stress was the consistent predictor of how many eating changes a student reported.

Table 4: Association between academic stress and each eating outcome: crude, age-adjusted and multivariable estimates.

Eating outcome	% Yes, high stress (n = 448)	% Yes, others (n = 552)	Crude OR (95% CI)	Age-adjusted OR, high vs other (95% CI)	Age-adjusted OR per stress item (95% CI)	Model with all four stress items: pseudo-R2; LR p
Q7 Skip meals	27.7	20.7	1.47 (1.10 to 1.97)	1.39 (1.00 to 1.93)	1.11 (0.95 to 1.30)	0.006; 0.22
Q8 Amount eaten changes	38.8	33.5	1.26 (0.97 to 1.63)	1.13 (0.85 to 1.51)	1.07 (0.93 to 1.23)	0.007; 0.13
Q9 Weight/appearance thoughts	24.3	21.6	1.17 (0.87 to 1.57)	1.07 (0.77 to 1.48)	1.00 (0.85 to 1.17)	0.003; 0.62
Q10 Eating habits influenced	35.3	36.4	0.95 (0.73 to 1.23)	0.84 (0.63 to 1.13)	0.93 (0.81 to 1.07)	0.005; 0.28
Any eating change	77.2	73.0	1.25 (0.94 to 1.68)	1.09 (0.78 to 1.50)	0.99 (0.85 to 1.15)	0.010; 0.044
Multiple eating changes (2+)	36.6	32.1	1.22 (0.94 to 1.59)	1.07 (0.80 to 1.44)	1.00 (0.87 to 1.15)	0.007; 0.099

OR, odds ratio; CI, confidence interval; LR p, likelihood-ratio test of the full model against an intercept-only model. All adjusted models include age in years.

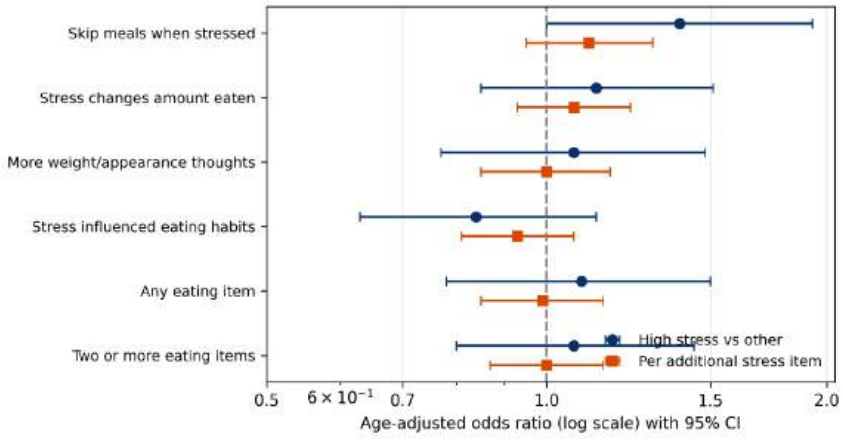


Fig 3: Age-adjusted odds ratios with 95% confidence intervals for each eating outcome, comparing high-stress students with others (circles) and per additional stress item endorsed (squares). The dashed line marks no association.

Correlation, effect modification and the joint distribution

The Spearman correlation between the stress count and the eating count was 0.06 (bootstrap 95% CI -0.003 to 0.122, p = 0.057). Once the linear effect of age was removed from both counts, the partial correlation was 0.00 (p = 0.96): the small crude correlation was entirely explained by the fact that older students reported more of both. The joint distribution of the two

counts (Table 5) illustrates this. Among students endorsing all four stress items, 23.5% reported no eating change and 15.9% reported three or four, compared with 26.8% and 4.9% among those endorsing none; the shift is visible but slight, and the modal response at every level of stress was a single eating change. The high-stress by age-band interaction for meal skipping was not significant (likelihood-ratio p = 0.98), and the association was similar in each band (ORs 1.39, 1.46 and 1.37), so the stress-meal skipping link, though modest, was consistent from age 10 to 18. Fig 4 shows the proportion endorsing each eating item at each level of the stress count.

Table 5: Joint distribution of the stress count and the eating count (number of students).

Stress items endorsed	**Eating items: 0**	**1**	**2**	**3**	**4**	**Total**	**% with 2+ eating changes**
0	11	16	12	2	0	41	34.1
1	46	74	44	12	0	176	31.8
2	92	136	85	19	3	335	31.9
3	62	114	80	20	2	278	36.7
4	40	68	35	23	4	170	36.5
Total	251	408	256	76	9	1,000	34.1

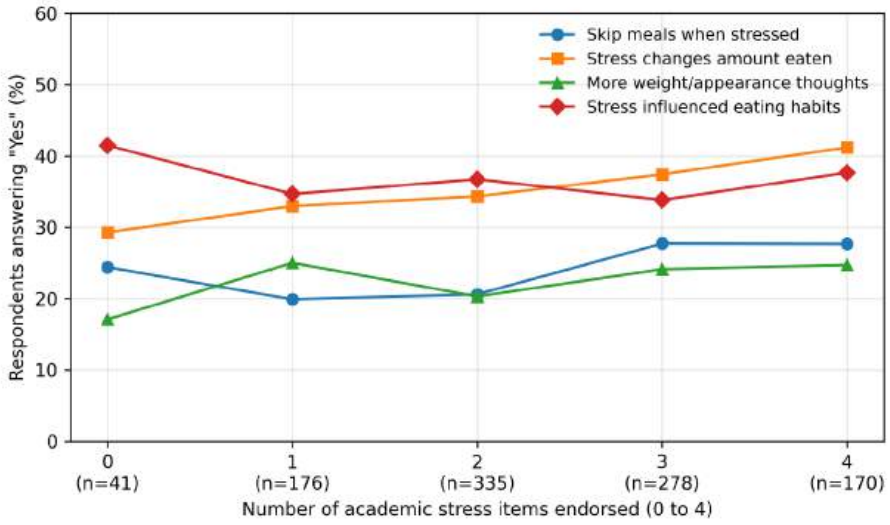


Fig 4: Proportion of students answering "Yes" to each stress-related eating item, by the number of academic stress items endorsed (0 to 4). Group sizes are shown beneath each level.

The combined pattern, sensitivity analyses and power

A total of 164 students (16.4%, 95% CI 14.2 to 18.8) showed the combined pattern of high academic stress together with two or more eating changes. This is close to the 153 expected if the two were independent, consistent with an absence of clustering, but the prevalence of the combined pattern rose fivefold with age, from 5.6% of 10 to 12-year-olds to 14.8% of 13 to

15-year-olds and 27.5% of 16 to 18-year-olds, driven almost entirely by the rise in stress. Restricting the analysis to the 876 students whose age and grade were in the expected relation to each other did not change any conclusion: the correlation between age and the stress count was 0.47, the age-partialled correlation between the stress and eating counts was 0.02 ($p = 0.64$), the age-adjusted OR for meal skipping among high-stress students was 1.48 (95% CI 1.05 to 2.09, $p = 0.024$), and the ORs for the other three eating items were 1.15, 1.07 and 0.83 (all $p > 0.2$). Excluding the 213 students aged 10 to 11 also left the conclusions unchanged: the age-adjusted OR per stress item for meal skipping was 1.18 ($p = 0.056$) and for the other outcomes 0.91 to 1.12 (all $p > 0.13$). Post-hoc power calculations showed that the comparison of 448 high-stress with 552 other students had 78% to 88% power to detect an odds ratio of 1.5 at the observed outcome prevalences, and 61% to 74% power for an odds ratio of 1.4. The study was therefore adequately powered to detect moderate associations, and the null findings for most outcomes are unlikely to reflect insufficient sample size, though very small effects (OR below about 1.3) could have been missed.

Discussion

Three findings stand out. First, academic stress in this sample was both very common and strongly age-dependent: pressure for grades was reported by half of 10 to 12-year-olds and by 85% of 16 to 18-year-olds, high academic stress rose from fewer than one in ten 10-year-olds to three in four 18-year-olds, and the stress count climbed in every single year of age with no plateau before 17. Second, stress-related eating changes were reported by a substantial minority at every age but rose only slightly with age, so that a primary school student and a senior secondary student were nearly equally likely to say they skipped meals or thought more about their weight when stressed. Third, and contrary to the initial hypothesis, there was little evidence of a meaningful association between the two sets of reports once age was accounted for. The partial correlation between the stress and eating counts was zero, none of the 16 item-level associations survived correction for multiple testing, no individual stress indicator predicted eating in multivariable models, and the only behaviour that showed a consistent, if modest, relationship with stress was skipping meals.

The age gradient in academic stress

The steepness of the stress gradient is the most striking descriptive result. Cramer's V of 0.43 for high stress against age band is a large effect by conventional standards, and the trajectory in Fig 2 shows a near-linear climb from age 10 to age 17 rather than a step at any single age. This pattern is consistent with the accounts of Verma, Sharma and Larson and of Deb, Strodl and Sun, in which schoolwork is a persistent daily source of negative affect rather than only an examination-time one, and it extends those accounts downward: a third of 10 to 12-year-olds already felt overwhelmed by workload. The practical implication is that interventions timed for the final two years arrive after most of the increase has already taken place. If schools wish to reduce academic stress, the years from age 12 to age 16, when high

stress rose from about a quarter to about two-thirds of students, appear to be the period of fastest change.

Why stress and eating were only weakly linked

The weakness of the association between stress and eating, in a sample large enough to detect moderate effects, calls for explanation. A weak association in these data does not show that academic stress has no effect on eating; it shows that, with these items and this design, any effect was too small or too inconsistent to detect. Three explanations are likely to operate together. The first is measurement. The eating items were single yes/no questions, and the two-directional nature of stress eating means that "stress changes how much I eat" is endorsed by students who eat more and by students who eat less (Oliver et al.; Torres and Nowson). If these two groups differ in how stress affects them, and if the prevalence of each rises and falls in different ways, their combined association with stress will be diluted toward zero. The near-chance agreement between Q8 and Q10, two items that on their face ask almost the same thing, further suggests that respondents interpreted the items differently or answered them inconsistently, which would attenuate any true relationship. The KR-20 values confirm that neither item set behaved as a scale.

The second explanation is confounding by age, which this study was able to address directly. Because stress rose steeply with age and eating changes rose slightly, the crude correlation of 0.06 was produced entirely by their shared dependence on age; after partialling age out it disappeared. Studies that compare stress and eating without adjusting for age in a sample spanning several school years risk reporting an association that is really a developmental gradient.

The third explanation is that, in this population, stress-related eating may be a relatively stable trait or a response to stress in general rather than to academic stress specifically. At least one eating item was already endorsed by 71% of 10 to 12-year-olds, at an age when few reported high academic stress, and their prevalence changed little thereafter. This is consistent with Hill and colleagues' finding that emotional eating in youth relates to trait-level characteristics, and with Roemmich, Wright and Epstein's demonstration in children that dietary restraint, not stress exposure alone, determines who snacks under stress. Academic stress may simply be one of many triggers acting on a pre-existing tendency, so that the level of academic stress adds little predictive information once the tendency is present.

Meal skipping

Meal skipping was the partial exception. High-stress students had 39% higher age-adjusted odds of skipping meals, the estimate was similar at every age, and it strengthened slightly in the sensitivity analyses. The association is modest, the lower confidence limit touched 1.00, and the cross-sectional design cannot show that stress causes meal skipping rather than the reverse or that both reflect a third factor; it should be read as a suggestive finding that warrants replication. Two mechanisms make this plausible. Acute stress suppresses appetite through the

sympathetic and corticotropin-releasing hormone response (Adam and Epel), and students under time pressure may deliberately trade meals for study time. Unlike the other items, "I skip meals" describes a discrete, easily recalled behaviour with a clear direction, which may also explain why it alone showed a signal. Meal skipping in adolescence predicts poorer diet quality, weight gain and later disordered eating (Neumark-Sztainer et al.), so even a modest association with academic stress is relevant to schools.

Body image

Thinking more about weight or appearance under stress was reported by about one in five students at every age and was unrelated to the stress count in every model. This is best read as evidence that body image concerns in this age range have drivers largely separate from academic pressure, such as peer comparison, media exposure and puberty (Stice et al.), and not as evidence that academic stress either protects against or promotes body dissatisfaction. Because no sex variable was collected, the study could not examine the difference between girls and boys that dominates this literature.

Implications

Two practical points follow. First, stress reduction in schools is justified across the whole age range surveyed and should scale up from about age 12 onward, not begin in the final examination years. Second, blanket messages about "stress eating" are unlikely to be well targeted, because the eating changes that accompany stress differ by behaviour and do not rise together. Attention to regular meals, for example by protecting meal breaks during examination periods and discouraging students from trading meals for study time, would address the one behaviour that showed a modest association with academic stress. Future studies should use validated instruments that record the direction of change in intake, collect sex and body composition, measure timing relative to examinations, and follow students over time to establish whether academic stress precedes changes in eating.

Study strengths and limitations

The study's strengths are its size, the breadth of the age range covered with an identical instrument, the absence of missing data, and an analytic approach that tested the stress-eating relationship in several complementary ways, including correction for multiple comparisons, adjustment for age, multivariable models, count models, interaction tests, sensitivity analyses and power calculations, so that the weak association can be reported with confidence rather than as a failure to look.

The limitations are equally important. The design was cross-sectional, so no causal direction can be inferred. All data were self-reported and unverified. The questionnaire was purpose-built, unvalidated and limited to yes/no answers, and its low internal consistency means that the stress and eating counts are tallies rather than measures of underlying constructs; the near-chance agreement between two similar eating items suggests that some students read these

items differently or answered them inconsistently, which would bias any true stress-eating association toward zero. The youngest respondents, aged 10 to 11, may not have understood the items fully, although excluding them did not change the results. The study was not reviewed by an ethics committee, and parental consent was obtained through a statement on the form rather than through signed documentation, so it could not be verified for each respondent; although the survey was anonymous, voluntary and low-risk, formal review and documented guardian consent would be expected in a study of minors conducted under institutional auspices, and future work should obtain them. For 12.4% of respondents the reported age and grade were further apart than usual, which may reflect grade repetition, late entry, or simple reporting error; because the results were unchanged when these students were excluded, age was retained as the developmental variable and grade was not analysed separately. No information was collected on sex, socioeconomic status, body mass index, physical activity, sleep or the timing of the survey relative to examinations, any of which could confound or modify the relationships studied. Sampling was by convenience and open circulation across schools in a single Indian state; because the number of students who received the link was not recorded, no response rate can be given, and the 1,000 respondents should not be taken as representative of students in Andhra Pradesh or elsewhere. Students who chose to respond may differ from those who did not, for example in how much stress they felt, and prevalence figures in particular should be read as descriptive of this sample rather than of the wider population. Finally, with 1,000 respondents, small differences reach statistical significance, and several significant age differences in eating were of small absolute size; conversely, the study could have missed very small true associations between stress and eating.

Conclusion

In this survey of 1,000 school students aged 10 to 18, academic stress was pervasive and rose steeply and steadily across the school years, while stress-related changes in eating were reported by a quarter to a third of students at every age and rose only slightly. Once age was accounted for, there was little evidence that the level of academic stress a student reported was associated with whether that student reported eating changes, with the single, modest and suggestive exception of meal skipping, which was somewhat more common among high-stress students at every age. These results caution against the assumption that academic stress and disordered eating rise together, and they point to two concrete targets for schools: reducing academic pressure from early adolescence onward, and protecting regular meals during periods of high academic demand.

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Analysis of Sciatic Nerve Disease By Niyati Deshmukh

Abstract

This review summarizes the impact sciatic nerve disease has on an individual's daily life. It describes conditions, risk factors, causes, symptoms, and treatment. Studies indicate that a herniated disc or nerve compression leads to vast damage in a person's normal body functions, highlighting the notable change in lifestyle that would occur once receiving a diagnosis. A preliminary review of available literature was conducted using credible online sources and authoritative research to compile current statistics and findings. Understanding the procedures and management strategies will improve patient outcomes and guide future care.

Introduction

The subject of sciatica (lumbar radiculopathy) is found to be very common within 40% of people in the U.S. who experience some form of sciatica in their lifetime (Cleveland Clinic; Johns Hopkins Medicine; Valat et al.; NCBI Bookshelf). According to Cleveland Clinic and Atlantic Brain and Spine, sciatica is a nerve pain which causes intense irritation or pinching to the sciatic nerve. It is most described as sharp acute pain that can feel like an electric shock-like jolt, or burning sensations, and these symptoms usually only affect one side of the body (Cleveland Clinic; Atlantic Brain and Spine; Mayo Clinic; Hospital for Special Surgery). Sitting, coughing, or sneezing can aggravate the pain (Mayo Clinic; Cleveland Clinic). The sciatic nerve, the longest nerve in the body, originates from spinal nerve roots L4 through S3 from the back of the pelvis, along the back of the thigh, and continuing to the ankle and toe of your foot (Johns Hopkins Medicine; UTHealth Neurosciences; NCBI Bookshelf). It is essential to be aware of this topic due to the lack of awareness from healthcare and media we receive about how much of a catastrophic and chronic impact Sciatica could have in our near future if we do not maintain the healthy habits necessary to prevent its arrival.

Methods

Literature Search Strategy

A literature review was conducted to synthesize information on how Sciatica affects individuals' physical activity, occupational responsibilities, mobility, and quality of life. This meant focusing on how the symptoms of sciatica would have limited a patient's ability to participate in their most appreciated hobbies, being able to drive, their jobs, and even meal preparation.

Database Selection

A preliminary review of available literature was conducted by using Google Scholar, PubMed, ScienceDirect, Oxford Academic, and BMJ Open to identify credible sources related to sciatica. Keywords included "sciatica," "lumbar radiculopathy," "sciatic nerve pain," "quality of life," "treatment outcomes," and "daily functional limitations." Additional information was

obtained by authoritative medical resources, including Cleveland Clinic, Mayo Clinic, Johns Hopkins Medicine, Yale Medicine, Hospital for Special Surgery, Harvard Health Publishing, WebMD, UT Health Science Center at Houston, and NCBI Bookshelf.

Data Analysis

Literature analysis took place to evaluate differences among studies, age trends, symptoms, treatment effectiveness, and impacts on daily functioning. Statistical data from relevant studies were reviewed to assess prevalence rates of people affected by Sciatica.

Results

Risk factors

According to Mayo Clinic sources, primary risk factors for sciatica include: people between the ages of 20-50 since bone spurs develop more commonly as people age. Individuals who sit for prolonged amounts of time are more at risk for a herniated disk due to the lack of movement and time given when sitting still allows for irritation in the buttock area of the sciatic nerve. Jobs that require heavy lifting/backtwisting are additionally more at risk for a herniated disk. People of the overweight demographic are more at risk due to the irritation to the spine caused by excessive weight. Diabetics are exhaustively burdened by the way that diabetes affects the way that the body uses blood sugar and can cause extreme nerve damage (Mayo Clinic; Cleveland Clinic; Hospital for Special Surgery; Harvard Health Publishing).

Causes of Sciatica

Complications of osteoarthritis, a type of arthritis associated with inflammation of the tissues in the joints can be a leading cause to damage in the sciatic nerve (Yale Medicine). Other spinal disorders include spondylolisthesis, spinal stenosis, and spinal tumors, which may compress the sciatic nerve. Additionally, physical trauma such as sports injuries, blunt force trauma, and pelvic fractures, along with other conditions including pregnancy, Paget's disease, can increase the risk of sciatica (Mayo Clinic; Yale Medicine; Valat et al.; ScienceDirect; NCBI Bookshelf).

Symptoms of sciatica

According to Hospital for Special Surgery sources, symptoms of sciatica include dull, aching, shooting or "burning" pain that starts in the lower back and/or buttock and radiates down one of the legs. It originates from an irritated nerve which can stem from several nerves in the lumbar (lower spine) region. Back pain accompanied by leg pain is the key symptom that suggests you have sciatica rather than another type of back pain. Neurological symptoms such as numbness, radiating and tingling from the thigh through ankles and toes, intense hot/cold sensations, electrical sensations, or others. Muscle weakness and altered reflexes may be signs of sciatica due to the changes in inhabited body functions. Symptoms may worsen with certain motions such as bending or twisting the low back or raising the painful leg. Symptoms may also

worsen with coughing, sneezing, bowel movement, or straining since all actions trigger the sciatic nerve (Hospital for Special Surgery; Cleveland Clinic; Mayo Clinic; Atlantic Brain and Spine; WebMD).

Treatment

Atlantic Brain and Spine states that sciatica usually responds well to conservative treatments including hot and cold packs, stretching, exercise, and over-the-counter pain medications. Physical therapy is often recommended if a herniated disc is causing your sciatica (Atlantic Brain and Spine; Physical Therapy Journal; PubMed). A doctor may also prescribe pain medications or an epidural steroid injection. More aggressive treatments are usually only recommended when conservative treatment doesn't relieve your symptoms. Surgery for sciatica is usually performed in cases where symptoms have not resolved after 6 weeks of treatment. When a herniated disc is the cause of the pain, a doctor will perform surgery to remove part of the herniated disc that is pressing on the sciatic nerve. This will decompress the sciatic nerve root and reduce the risk that the disc may herniate again. In rare cases, if sciatica goes untreated early on, it can result in permanent nerve damage and chronic pain (Atlantic Brain and Spine; Cleveland Clinic; Johns Hopkins Medicine; Physical Therapy Journal).

Discussion

The objective of this review was to examine risk factors, causes, symptoms, and treatments of sciatica. This medical literature consistently specifies that sciatica is primarily caused by spinal disorders, injuries, pregnancy, improper care for the body, and tissue complications (Mayo Clinic; Yale Medicine; Valat et al.; NCBI Bookshelf). While these sources agree on the primary causes of sciatica, recoveries and treatments vary based on severity. For example, many mild cases may improve within approximately six-weeks of conservative treatment,whereas more severe cases of a herniated disk may require a surgical intervention followed by an extensive recovery process (Atlantic Brain and Spine; Peul et al.; Valat et al.). Medical sources persistently describe the pain as pinching, sharp and acute, numbness, tingling, burning, and an electric shock-like jolt (Cleveland Clinic; Mayo Clinic; Yale Medicine; Hospital for Special Surgery; Atlantic Brain and Spine; WebMD). These findings suggest the significant physical limitations and daily life disruptions that sciatica has on many individuals. Highlighting the importance of preventative measures, which can include maintaining a healthy weight, doing regular core and cardio exercises, practicing good posture,weight lifting, refraining from smoking, consistent stretching, and avoiding prolonged sitting, are highly recommended to reduce the risk of developing sciatica (Cleveland Clinic; Mayo Clinic; Harvard Health Publishing; Hospital for Special Surgery). In the case of an individual having a high intensity job, it is important to maintain adequate core, hip, and knee strength (Hospital for Special Surgery; Physical Therapy Journal). However, current research is limited by gaps in treatment evidence, primary care approaches, long term outcomes, diagnostic standardization, and prognostic factors (Physical Therapy Journal; Valat et al.; BMJ Open; PubMed). Future research should focus on these areas topics to improve the understanding and treatment of sciatica.

Conclusion

To conclude the entity of sciatic nerve disease, sciatica is found to be extremely common and harmful towards many individuals. It takes a major toll on diminishing a person's daily life and activities, altering their livelihood for the worse (BMJ Open; Physical Therapy Journal; Valat et al.). Many stay untreated with sciatica due to the lack of knowledge from the media and insufficient communication from health care professionals. Treatments can take extensive amounts of time to become effective, due to the incredibly prolonged recovery process even after surgery. It can later succumb to being required to get physical therapy and spinal injections (Cleveland Clinic; Atlantic Brain and Spine; Peul et al.). It remains a significant health problem to many, and more awareness should be spread relating to it, such as being educated on healthy habits of how to prevent it while reiterating how incredibly your livelihood would be affected. Overall, a deeper understanding of this condition has the potential to greatly improve patient care and quality of life, since the longer a patient stays untreated, the condition will only continue to exponentially worsen and damage the nerve.

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I would like to express my gratitude to my mother who has experienced extreme hardships stemming from sciatica in her everyday life for the past three years. Her resilience and perseverance to still be there for me everyday despite how difficult living her life has become has inspired me to conduct this review and deepened my understanding of the impact of this condition. This work is dedicated to her and all other individuals affected by sciatica, in hopes of contributing to better awareness and patient care.

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Understanding Fluctuations in Self-reported NSQ Scores and Perceived Stress Among Six Registered Nurses in Pakistan: A Pilot Study By Eshal Amin

Abstract

Healthcare professionals, and nurses in particular, experience elevated occupational stress driven by heavy workloads and the emotionally demanding nature of patient care, with chronic exposure linked to cardiovascular, immune, and cognitive impairment. However, how stress and stress-related self-reported neuroplastic symptoms fluctuate day to day across working and non-working conditions remains poorly understood. This study examined how workload affects nurses' perceived stress and self-reported neuroplastic symptom patterns on working and non-working days, and how sleep, workload, and shift demands interact. Six registered nurses working in inpatient hospital settings completed a repeated-measures design across three consecutive days, comprising two working days and one non-working day, using structured online questionnaires that captured baseline demographics and stress via the Perceived Stress Scale, as well as pre- and post-shift measures of momentary stress and the Neuroplastic Symptom Questionnaire (NSQ). Perceived stress increased slightly from pre- to post-assessment across both working days and the off day, with the same mean increase observed across all three conditions. NSQ symptom scores decreased across all three days, with the largest descriptive reduction observed on the off day. Given the small sample size, fixed order of assessment days, and limitations associated with repeated NSQ administration, these findings should be interpreted as exploratory and do not provide evidence of occupational or neuroplastic recovery effects.

Keywords Nurses, self-reported symptoms, stress, workload and Pakistan

Introduction

Healthcare professionals have consistently been reported to experience high levels of stress, linked to organisational factors like heavy workloads and fast-paced environments where mistakes can have serious consequences. Nurses, in particular, perform a wide range of tasks at work, which can be stressful; some tasks are perceived as more stressful than others. Occupational stress in the nursing profession has been widely researched over the past two decades.

Several studies have explored how workplace stressors affect the health and overall well-being of hospital nurses. Hovland et al. found that direct and indirect patient care tasks are demanding and associated with higher levels of emotional distress among nurses and physicians, highlighting the stressful nature of nursing work. Building on this, research has also examined the physiological consequences of extended shift patterns. A cross-sectional study conducted in the United States found that working consecutive shifts elevated stress and fatigue in nurses, with cortisol levels following a predictable daily pattern, peaking in the morning and declining by evening, suggesting that repeated shift exposure further strains the body's stress response

(Cockerham et al.). These cumulative effects of shift work are concerning given that fatigue has been shown to increase progressively over consecutive shifts, especially among night-shift nurses. At the same time, social support has been identified as a key buffer against these effects (Cockerham et al.).

In many cases, however, healthcare workers do not receive adequate institutional support, which can accelerate this trajectory from stress to burnout. Although burnout has traditionally been viewed as a stable condition, there is growing recognition of the need to examine it as a dynamic, fluctuating phenomenon (Bakker and Costa). Since burnout is subjective, individuals are likely to assess it based on their daily experiences and perceptions, which are influenced by numerous day-to-day factors. As a result, burnout may vary frequently, reflecting the natural ups and downs people experience in their working lives (Martínez-Zaragoza et al.).

When burnout persists without adequate intervention, it can transition into chronic, long-term stress, with serious consequences for both physical and mental health. Long-term stress can seriously affect the physical and mental health of healthcare workers. Studies have linked chronic stress to problems such as heart disease, high blood pressure, sleep disorders, and reduced job performance (Alhajaji et al.). Stress can also increase the likelihood of mistakes in patient care, making it an important issue for both healthcare workers and healthcare systems (Kwak et al.).

Research has shown that psychological stress affects many body systems, including the cardiovascular, immune, and metabolic systems. Stress can alter immune responses and increase inflammation, with studies linking stress to inflammatory markers such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumour necrosis factor (TNF-α) (Steptoe et al.). Other factors, such as low socioeconomic status, work stress, social isolation, and depression, have also been connected to increased inflammation (Miller et al.). Both human and animal studies confirm that the immune system responds rapidly to psychological stress, altering the number and activity of immune cells and impairing normal immune function (Segerstrom and Miller; Dhabhar).

Beyond these physical outcomes, prolonged stress has also been associated with structural and functional changes in brain regions involved in memory, emotional regulation, and stress responses. Chronic exposure to stress hormones such as cortisol may affect the hippocampus and prefrontal cortex, which are involved in learning, memory, decision-making, and emotional regulation, and influence amygdala activity associated with threat and emotional processing (McEwen). Stress-related changes may contribute to difficulties with cognitive functioning and emotional regulation. These effects may be particularly relevant in nursing, where sustained attention, decision-making, and emotional regulation are important when responding to demanding clinical environments.

Nurses are a vital part of the healthcare system, providing essential care and support to patients. However, the demanding nature of nursing can negatively affect nurses' mental health and wellbeing. Stress is a major issue among nursing staff in the UK, with reports showing that many nurses experience burnout, work-related illness, and thoughts of leaving their jobs (Royal College of Nursing). To reduce the impact of stress on nurses, it is important to identify the main

causes of stress and develop effective strategies to manage it. Addressing stress not only improves nurses' well-being but also helps maintain high-quality healthcare services for patients. The present study therefore aims to examine how shift work affects nurses' perceived stress across different working conditions. The primary research questions examined: (1) how stress levels vary between working and non-working days, (2) how pre-shift stress compares to post-shift stress on working days.

Methods

Experimental Design and Tools Used

A repeated-measures survey design was employed to assess within-person variations in stress across different time points and working conditions. By capturing data at multiple time points over three consecutive days, the design allows examination of stress as a dynamic, fluctuating experience rather than a fixed trait — an important consideration given that stress in nursing varies considerably with shift demands, workload, and daily recovery.

The study was carried out in two phases across three consecutive days. In Phase 1 (Days 1 and 2, working days), participants completed structured surveys immediately before and after their clinical shifts, capturing stress and symptom data under active occupational exposure. In Phase 2 (Day 3—non-working day), participants completed equivalent surveys at times aligned with their usual shift start and end times, enabling a temporally consistent comparison between working and non-working conditions.

Data were collected using structured online questionnaires administered at multiple time points. Standardised instruments included:

Perceived stress scale: Stress was assessed using the Perceived Stress Scale (PSS), developed by Cohen, Kamarck, and Mermelstein (1983). The PSS is a widely used self-report instrument designed to measure the degree to which individuals appraise situations in their lives as unpredictable, uncontrollable, and overloaded over the past month. Respondents rate each item on a 5-point Likert scale ranging from 0 (never) to 4 (very often), with several positively worded items reverse-scored before summing. Total scores are obtained by summing all item responses; higher scores reflect greater perceived stress, and lower scores reflect lower perceived stress. The scale has demonstrated good internal consistency and validity across diverse populations, making it one of the most extensively used measures of psychological stress in health-related research (Cohen et al.).

A 5-point Likert scale to assess **momentary stress levels** before and after each shift (1 = Not at all stressed; 5 = Extremely stressed). This measure enabled tracking of acute, real-time fluctuations in stress within and across shifts.

The Neuroplastic Symptom Questionnaire (NSQ): The NSQ was administered in both the pre- and post-shift surveys on all three days. Neuroplastic symptom patterns were assessed using the Neuroplastic Symptom Questionnaire (NSQ), developed by Dr Matt McClanahan of the Centre for Insight Medicine (2022). The NSQ is a 13-item yes/no questionnaire designed to

help distinguish symptoms arising from neuroplastic sensitisation of the nervous system, characterised by variability, stress-reactivity, fear-amplification, and inconsistent triggering, from symptoms attributable to purely structural or pathological causes. The nurses answered "yes" or "no" to each question. The NSQ is interpreted based on the number and pattern of "yes" responses: a higher number of ‘yes’ answers, particularly to the first five items, which are considered the most diagnostic, suggests a greater likelihood that the individual's symptoms are neuroplastic in origin. That means they are amplified or perpetuated by nervous system sensitisation, stress, and fear rather than by tissue damage or disease. For this pilot study, the number of symptoms endorsed was calculated by coding each ‘yes’ as 1 and each ‘no’ as 0, then summing the items for each administration. This derived variable is referred to throughout the study as the NSQ symptom scores. Higher counts indicate a greater number of symptoms and lower counts indicate fewer symptoms endorsed. This count is an exploratory descriptive measure; changes in the count were not interpreted as direct evidence of neuroplastic change.

Sample Characteristics

The study included registered nurses working in clinical healthcare settings. Participants were drawn from a range of hospital departments and worked across varied shift schedules. The sample reflected diversity across several demographic dimensions relevant to research, including gender, marital status, parental status, and years of clinical experience. The sample comprised 6 nurses, of whom 83.3% were females and 16.7% were males. 83.3% of respondents were between 18 and 25 years old, while 16.7% were between 26 and 35 years old. 83.3% of respondents held the role of Staff Nurse (Junior), while 16.7% were registered beginner-level nurses. All respondents (100%) reported having 1-3 years of experience. In terms of marital status, 83.3% of respondents were single, while 16.7% were married; correspondingly, 83.3% reported having no children, while 16.7% reported having children. All respondents (100%) worked in inpatient settings, of whom 83.3% worked day shifts and 16.7% worked night shifts.

Recruitment and Inclusion Criteria

Participants were recruited using convenience sampling from clinical settings. This approach was selected given practical constraints around access to healthcare professionals and the time-intensive nature of the repeated-measures protocol. Inclusion criteria required that participants be registered nurses with at least 1 year of professional clinical experience. Only nurses actively engaged in shift-based clinical duties were eligible to participate, as this ensured that all participants were exposed to both working and non-working day conditions throughout the data collection period — a prerequisite for the repeated-measures comparison central to the study design.

Ethical Considerations

All participants provided written informed consent before participation, having been fully briefed on the study's purpose, procedures, and their rights as participants. Confidentiality and

pseudonymisation were maintained throughout the study by assigning each participant a unique pseudonymised code consisting of their initials and date of birth (e.g., EK12). This code was used across all survey responses in place of identifying information. Participants were informed that their responses would be used solely for research purposes and would not be shared with their employers or clinical supervisors. Participation was entirely voluntary, and nurses were explicitly informed of their right to withdraw at any stage of the study without consequence or need for justification.

Data Collection Procedure

Data collection took place over three consecutive days following a structured repeated-measures protocol. Before the three-day data collection window began, all participants completed a baseline survey capturing demographic information and their baseline perceived stress using the full Perceived Stress Scale (PSS). This baseline survey established each participant's pre-study stress reference point and provided contextual information for interpreting subsequent daily fluctuations.

Phase 1: Working Days (Days 1 and 2): On each working day, participants completed a pre-shift survey within 30 minutes before their shift began. This survey assessed sleep duration and quality from the preceding night, caffeine intake, expected shift start time, current stress level on a 5-point Likert scale, and the Neuroplastic Symptom Questionnaire (NSQ). A post-shift survey was then completed within 60 minutes of shift completion, measuring perceived shift demand, the number and duration of breaks taken, whether overtime was worked, post-shift stress level on a 5-point Likert scale, and NSQ responses. This pre-post structure allowed within-shift stress change to be quantified and shift-level factors (such as overtime and break frequency) to be examined as potential moderators of stress elevation.

Phase 2:Non-Working Day (Day 3): On the non-working day, participants completed equivalent morning and evening surveys at times aligned with their usual shift start and end times. This temporal alignment was used to reduce potential differences attributable to the timing of assessments between working and non-working days. The survey content on Day 3 mirrored that of the working-day pre- and post-shift surveys, except shift-specific questions (e.g., overtime, shift demand), which were adapted for the non-working context. Together, the three-day protocol produced a rich within-person dataset capturing stress trajectories across varied working conditions.

Results

Table 1 summarises the baseline demographic and lifestyle characteristics of the six nurse participants before the onset of the three-day monitoring protocol. Baseline perceived stress scores ranged from 15 to 19, with a mean of approximately 17.2. Weekly work hours were largely uniform (42 hours) across five of the six participants, with nurse 1 reporting a comparatively lighter schedule of 30 hours. Sleep duration varied considerably across participants, ranging from 4.5 to 8.8 hours, with nurses 4–6 reporting the shortest sleep durations

(4.5- 6.5 hours) alongside the lowest self-rated sleep quality scores (3 out of a 5-point scale). Caffeine intake was recorded as a binary variable, with four of the six nurses reporting consumption. Two participants reported overtime, while four reported taking a 1-hour break during their shift. Taken together, these baseline figures and weekly work hours/sleep deprivation and perceived stress varied across participants. They were presented descriptively; no inferential relationships between lifestyle, work characteristics, and perceived stress were tested due to the small sample size.

Table 1: Lifestyle, baseline stress and work characteristics of the registered nurse participants (n= 6).

Nurses	Baseline perceived stress score	Weekly work hours	Sleep hours	Sleep quality	Caffeine intake	Over time	Breaks
Nurse 1	15	30	8.2	5	0	1.5	1
Nurse 2	16	42	7.8	4	1	0	0
Nurse 3	16	42	8.8	4	1	0	0
Nurse 4	19	42	6.5	3	0	1.5	1
Nurse 5	18	42	4.5	3	1	0	1
Nurse 6	19	42	4.5	3	1	0	1

Table 2 presents descriptive statistics for pre- and post-shift perceived stress and NSQ response across the two workdays and 1 off day. Mean perceived stress increased slightly from pre- to post-shift on workday 1, workday 2, and the off day, with the same mean change (+0.17) observed across all three days. This suggests that the findings did not indicate an increase in stress on workdays compared with the off-day. NSQ responses decrease from pre- to post-assessment across all three days, with mean changes of -0.83 on workday 1, -0.33 on workday 2, and -2.17 on the off day. This pattern may be due to a small sample size, the fixed order of assessment days, and concerns regarding repeated measurement of the NSQ responses. These patterns are presented descriptively and should not be interpreted as evidence of occupational effects or neuroplastic change.

Table 2: Descriptive statistics for pre- and post- assessment stress and NSQ responses across workdays and the off days (n=6)

	Pre Mean (SD)	Post Mean (SD)	Mean Change
Workday stress 1	2.67 (1.51)	2.83 (1.72)	0.17
Workday stress 2	2.50 (1.52)	2.67 (1.21)	0.17
Off day stress	3.00 (1.90)	3.17 (2.04)	0.17
Workday 1 NSQ	3.00 (2.90)	2.17 (3.49)	-0.83

Workday 2 NSQ	1.17 (1.83)	0.83 (1.60)	-0.33
Off day NSQ	2.67 (1.86)	0.50 (1.22)	-2.17

Figure 1 shows mean pre- and post- assessment stress scores across the three conditions. Mean stress increased slightly across all three assessment days, from 2.67 to 2.83 on workday 1, 2.50 to 2.67 on workday 2, and 3.00 to 3.17 on the off-day. The mean change was +0.17 for each condition, depicting no difference in the magnitude of stress between workdays.

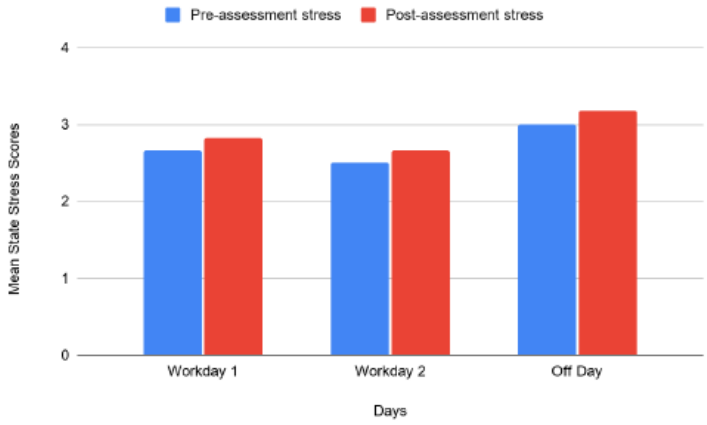


Figure 1: Graphical representation of mean pre- and post- assessment stress scores across workdays and off-day.

Figure 2 shows mean pre- and post-assessment NSQ symptom scores across the three days. Mean symptom decreased from 3.00 to 2.17 on workday one (mean change= -0.83), from 1.17 to 0.83 on workday 2 (mean change= -0.33), and from 2.67 to 0.50 on the off-day (mean change= -2.17). Descriptively, the largest pre-post reduction is observed on the off-day. However, pre-assessment NSQ responses varied across assessment days, especially between workday 1 and workday 2. Given this variability, the small sample size, and repeated measurement of the NSQ, these differences should not be interpreted as evidence of neuroplastic change or recovery.

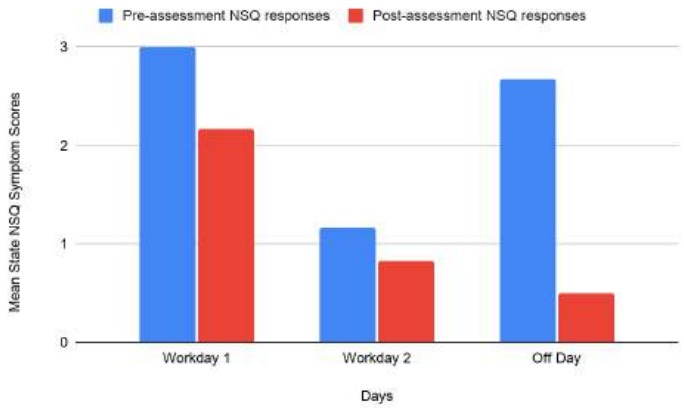


Figure 2: Graphical representation of mean pre- and post- assessment NSQ symptom scores across workdays and off-day.

Discussion

This exploratory pilot study examined within-day changes in perceived stress and self-reported symptoms, as captured by the NSQ, across two working days and one non-working day among six nurses. Perceived stress increased slightly from pre- to post-assessment on all three assessment days, with an identical mean change on Workday 1, Workday 2, and the off-day. Therefore, the descriptive findings do not indicate that perceived stress increased more during working shifts than on non-working days. Given the small sample size, these changes should be interpreted descriptively rather than as evidence of an occupational effect.

The presence of stress among nurses is nevertheless consistent with the wider literature showing that nursing work can involve substantial occupational stress. El Keshky et al. reported that nurses may experience considerable stress associated with workload, emotional demands, and working conditions. Similarly, previous research has linked occupational stress among nurses to factors such as workload, shift patterns, and inadequate recovery opportunities (Alenezi et al.). These studies provide context for examining short-term stress fluctuations among nurses, although the present pilot study does not establish that occupational exposure caused the observed changes.

A different descriptive pattern was observed for NSQ symptom responses. Mean scores decreased on Workday 1, Workday 2, and on the off-day. Thus, the largest pre–post reduction occurred on the off-day. However, substantial variability was present before the daily assessments, particularly between the Workday 1 and Workday 2 pre-assessment NSQ values. This variability makes it difficult to attribute subsequent changes specifically to working or non-working conditions.

Chronic stress has been associated with alterations in neural systems involved in stress regulation, emotion, and cognition (McEwen and Morrison). However, these findings pertain to longer-term biological processes and should not be interpreted as evidence of neuroplastic changes during the short assessment periods used in the present study. The current study did not directly measure brain structure, neural activity, or biological markers of neuroplasticity. Instead, the NSQ was used to capture self-reported symptom patterns that may be relevant to nervous-system sensitisation.

The NSQ findings therefore require particular caution. The questionnaire includes items concerning symptom history and relatively stable symptom characteristics, meaning that some responses are not necessarily expected to change over the course of a single nursing shift. In addition, the exploratory symptom endorsement count used in this study should not be interpreted as a validated continuous measure of neuroplasticity. The observed pre–post differences could reflect day-to-day symptom variability, repeated administration, differences in interpretation of questionnaire items, or other unmeasured influences.

Sleep may also be relevant when considering nurses' experiences of stress and recovery. Previous research has demonstrated relationships between occupational stress, sleep quality, and fatigue among healthcare workers (Alenezi et al.). In the present study, sleep duration and quality varied across participants. However, because the sample included only six participants, these

descriptive patterns cannot establish that poorer sleep caused greater perceived stress. Similarly, conclusions about weekly work hours are not warranted, as five of the six participants reported working the same number of hours per week.

The larger descriptive reduction in NSQ symptom endorsements on the off-day should also not be interpreted as evidence that a day away from work produced recovery. Both working days occurred before the off-day, meaning that assessment condition was confounded with order. Participants had also completed the questionnaires repeatedly by the off-day. Consequently, the observed difference could reflect order effects, increased familiarity with the measures, natural day-to-day variability, or other factors rather than recovery specifically attributable to the absence of occupational demands.

Overall, these findings are better interpreted as evidence of variability in self-reported stress and symptom experiences rather than evidence of acute neuroplastic change. Nevertheless, the repeated-measures design demonstrates the feasibility of examining nurses at multiple points across working and non-working days. Future studies should recruit substantially larger samples, counterbalance the order of workdays and off-days, extend monitoring across multiple work-rest cycles, and incorporate validated state-sensitive psychological and physiological measures.

Implications and Limitations

Although the findings of this pilot study do not establish that occupational exposure caused changes in perceived stress or NSQ symptom endorsements, they highlight the potential value of monitoring nurses' stress experiences across working and non-working days. The repeated pre- and post-assessment approach used in this study may provide a useful framework for future research examining how nurses' stress changes across shifts and recovery periods. For hospital administrators and workforce planners, the findings reinforce the broader importance of considering opportunities for adequate rest and recovery in shift scheduling. However, recommendations regarding specific scheduling practices cannot be made from the present small pilot sample alone. Occupational health and workplace wellbeing programs may also benefit from further investigation into brief, validated measures of acute stress to help identify patterns of persistent work-related stress. At the individual level, stress-management and recovery-focused resources may help nurses manage occupational demands. However, the present study did not directly measure neuroplasticity or nervous-system sensitisation; therefore, the observed NSQ symptom endorsement patterns should not be interpreted as evidence that symptoms resulted from or recovered through neuroplastic mechanisms. Future research using larger samples, counterbalanced work and off-days, and validated state-sensitive measures is needed before clinical or workforce recommendations can be made.

This study has several important limitations. First, the sample was very small (n = 6) and was recruited through convenience sampling, limiting statistical power and generalisability to the wider nursing population. Second, the three-day observation period was too short to assess longer-term patterns or recovery across multiple work cycles. Third, the study relied entirely on self-report measures and did not include physiological indicators of stress. Importantly, the NSQ

was not designed to assess acute within-shift changes. Some NSQ items assess symptom history or relatively stable symptom characteristics that would not be expected to change meaningfully within several hours. Therefore, pre–post differences in NSQ symptom counts may reflect response variability or repeated administration rather than genuine short-term symptom change and should not be interpreted as evidence of neuroplastic change.

Furthermore, the two workdays always preceded the off-day, meaning that condition was confounded with assessment order and possible familiarity with the measures. Finally, one non-working day may not have been sufficient to capture recovery patterns. Future studies should use larger and more diverse samples, validated measures designed to assess acute changes, longer observation periods, and counterbalanced assessment schedules.

Conclusion

This exploratory pilot study examined changes in perceived stress and self-reported NSQ symptom endorsements across two working days and one non-working day among six nurses. Mean perceived stress increased slightly by the same amount (+0.17 points) across all three assessment days, providing no descriptive evidence that stress increased more during working shifts than on the off-day. NSQ symptom endorsements decreased across all three days, with the largest reduction observed on the off-day. However, substantial variation in pre-assessment NSQ responses, the fixed order of assessment days, repeated administration of the NSQ, and the small sample prevent this pattern from being interpreted as evidence of occupational or neuroplastic recovery.

The findings should therefore be regarded as preliminary and descriptive rather than causal. Nevertheless, the study demonstrates the feasibility of repeated within-day assessment of nurses' stress and symptom experiences. Future research using larger samples, counterbalanced working and non-working days, longer observation periods, and validated measures of acute stress and short-term symptom change is needed to determine whether occupational demands are systematically associated with nurses' day-to-day stress and recovery patterns.

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$PM_{2.5}$ Mediated Depression in Perimenopausal Women and Associated Methods of Mitigation By Pranit Bagchi

Introduction

More than 1 in 8 Americans aged 12 and older have experienced depression symptoms, according to data from the CDC collected between 2021 and 2023 (Amodio). This is nearly double the rate from a decade ago (Amodio). Five percent of adults suffer from depression, which is characterized by apathy and a predominantly sad and negative mood (Correia et al.). There are many problems associated with therapy, including the development of resistance and relapses after the end of therapy (Correia et al.). Major Depressive Disorder (MDD) is two times more prevalent in women of reproductive age compared to men (Liang et al.). Approximately 20% of women have experienced an MDD episode at some point (Liang et al.). Specifically, the rates of MDD and clinical elevations in depressive symptoms doubled and even tripled during the perimenopausal stage when compared to premenopausal and postmenopausal rates (Liang et al.). Despite these statistics, there is a lack of research concerning oxidative stress-induced depression in middle-aged women.

While the mechanisms underlying perimenopausal depression are complex and still not well-known, air pollution has been proposed to be a potential trigger. In recent years, one in ten deaths have been attributed to air pollution (Ritchie and Roser). Along with being one of the leading risk factors for death, air pollution is one of the main contributors to the global disease burden (Ritchie and Roser). The air pollution burden is usually higher in low- and middle-income countries (Ritchie and Roser). Deaths from indoor air pollution have seen a decline since 1990, but outdoor air pollution deaths have only decreased modestly (Ritchie and Roser).

No Causal Relationship Has Been Established Between $PM_{2.5}$ and the Onset of Depression

It is important to note that the findings presented in this article do not confirm a causal relationship between $PM_{2.5}$ exposure and the onset of depression. There is currently no sufficient evidence to support a causal relationship between particulate air pollution and cognitive decline, largely due to the fact that findings have not been consistent among the studies published (Gan et al.). However, findings from several studies have been presented to suggest a possible correlation between $PM_{2.5}$ and the onset of depression.

$PM_{2.5}$: Composition, Exposure, and Pathogenic Potential

$PM_{2.5}$ is a class of particles with an aerodynamic diameter less than 2.5 micrometers (State of Global Air). UFPs are often studied separately from $PM_{2.5}$ but still fall within the size range, having a diameter less than 0.1 micrometers (IQAir). $PM_{2.5}$ is especially prevalent in air pollution and is often a result of combustion, such as the incineration of coal and fossil fuels; vehicle emissions; industrial emissions; wildfires; and waste burning (State of Global Air; Calderón-Garcidueñas et al.). $PM_{2.5}$ is the pollutant causing the largest health impacts globally,

with effects including ischemic heart diseases, lung carcinoma, COPD, lower respiratory infection, type two diabetes, dementia, and adverse birth outcomes (State of Global Air). Globally, 99% of people are exposed to levels of $PM_{2.5}$ above the annual World Health Organization's (WHO) guideline level of five $\mu g/m^3$, and eleven percent of the global population lives in areas without national air quality standards (State of Global Air). Almost half of the U.S. population lives in areas with unhealthy air pollution levels (American Lung Association). Air pollution is the second leading risk factor for early death, with 7.9 million deaths in 2023 attributable to air pollution (State of Global Air). Of these, 4.9 million deaths were attributable to ambient $PM_{2.5}$ exposure (State of Global Air). The pathogenicity of $PM_{2.5}$ is exacerbated because of its small size, which allows easy transportability into the human body and presence in food and other products. UFPs exhibit high surface reactivity characterized by clustering of atoms at the surface, which allows novel chemical reactions differing from reactions in bulk form. These particles are significant due to large surface area, significant absorption efficiency, functionalization, and oxide-based magnetic properties (Calderón-Garcidueñas et al.). $PM_{2.5}$, particularly UFPs, may penetrate physiological barriers including vasodilated skin and the lung-tissue compartment and can cross the blood-brain barrier (BBB) (Calderón-Garcidueñas et al.; Salvi et al.; Kim et al.). UFPs can cross the alveolar and capillary barriers to reach the major capillary bed and also quickly reach systemic circulation through proposed mechanisms such as "red blood cell hitchhiking," (Calderón-Garcidueñas et al.).

Key Portals of Entry

Inhalation serves as a rapid portal of entry for environmental air pollution (Calderón-Garcidueñas et al.). The nasal olfactory pathway is an effective route for air pollution to reach the brain (Brugge et al.; Wang et al.). As seen in Figure 2, the olfactory nerve ends in extensive nasal vasculature (Samaridou and Alonso). The extensive nasal vasculature of the olfactory bulb facilitates the uptake of $PM_{2.5}$. The trigeminal nerve is an especially important transport mechanism—it dominates nose-to-brain delivery of intact polymeric nanoparticles. The ophthalmic and maxillary branches are key portals of entry, and the nerve also has a few fibers ending in the olfactory bulb (Calderón-Garcidueñas et al.). $PM_{2.5}$ can also enter through the gastrointestinal (GI) tract due to its presence in various foods, supplements, food packing, and toothpaste. Through these items, $PM_{2.5}$ can access the epithelium and damage the GI barrier integrity, which allows easy access to the enteric nervous system. The damage to the GI barrier starts at the epithelial and mucus layer, which can alter the microbiome directly and indirectly. This may lead to neuroinflammation and immune responses, further producing autoantibodies to tight junctions, which are critical to the passage of nanoparticles (NPs) through epithelial layers and basement membranes, ultimately reaching the submucosa, the nerve endings, and blood vessels (Calderón-Garcidueñas et al.).

Characteristics of Oxidative Stress

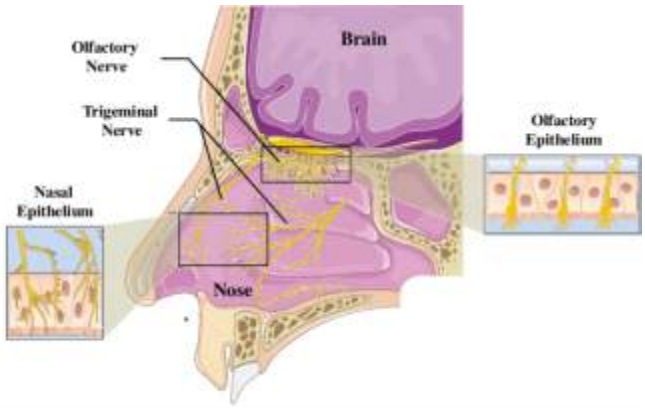


Figure 2: "Olfactory and Trigeminal nerve position in the nasal cavity" (Samaridou and Alonso)

Oxidative stress is an imbalance between reactive species and antioxidants, including both reactive species (ROS), also known as free radicals, and reactive nitrogen species (RNS) (Liang et al.; Cleveland Clinic). Specifically, oxidative stress is characterized by an accumulation of free radicals in the body, damaging cells and tissues (Cleveland Clinic). The main ROS species include superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen (Correia et al.). These may be generated through processes including immunity, apoptosis, protein phosphorylation, and other cellular signaling processes (Correia et al.). In the body, ROS are mainly produced in the mitochondria, peroxisomes, and the endoplasmic reticulum (ER) (Correia et al.). ROS, at low quantities, are important to maintaining homeostasis and cellular processes (Correia et al.). Oxidative stress can lead to the development of several chronic and degenerative conditions, such as cancer, cardiovascular disease, kidney disease, respiratory diseases, and neurological diseases (Cleveland Clinic). Free radicals are a natural byproduct of the conversion of food into energy. However, these radicals can accumulate in excess in response to triggers, including environmental toxins like $PM_{2.5}$. Free radicals are unstable molecules due to missing an electron (Cleveland Clinic). An accumulation of free radicals can cause them to take electrons from other molecules, ultimately damaging the body (Cleveland Clinic). The brain is especially susceptible to damage due to oxidative stress because of its high oxygen consumption and the presence of high levels of cellular lipids rich in saturated fatty acids (Liang et al.). ROS can lead to the oxidation and damage of lipids, DNA, and proteins in the brain (Liang et al.). Highly reactive species formed within ROS, including the oxide ion, can interact with nitric oxide to produce peroxynitrite (Liang et al.). Peroxynitrite can impair enzymatic function and tyrosine residues and can reduce the production of monoaminergic neurotransmitters and other aminergic compounds (Liang et al.).

$PM_{2.5}$ and Oxidative Stress Mechanisms

$PM_{2.5}$-induced Neuroinflammation and the Amplification of Oxidative Stress

$PM_{2.5}$ can trigger inflammatory mediators from primary entry organs or secondary deposition sites (Brugge et al.; Wang et al.). Pro-inflammatory cytokines are secreted by immune

cells, including CD4 T cells and macrophages, and act as chemical messengers to regulate inflammation in the human body (Liang et al.; ThermoFisher Scientific). Several interleukins, a type of cytokine, have been linked to systemic inflammation and subsequent oxidative stress and neurodegeneration as a result of $PM_{2.5}$ exposure, such as IL-15, IL-1β, IL-18, and IL-6 (Brugge et al.; Wang et al.). Other pro-inflammatory cytokines involved with this systemic inflammation include Tumor Necrosis Factor-Alpha (TNF-α), C-reactive protein (CRP), Epidermal Growth Factor (EGF), and CC-chemokine ligand (CCL) (Brugge et al.; Wang et al.; Ku et al.). These cytokines have several modes of transport to the brain. Some include passage through leaky circumventricular regions, such as the area postrema or the subfornical organ, in the BBB; transport through saturable transport molecules; activation of endothelial cells lining cerebral vasculature, which may then produce other inflammatory mediators; and binding to cytokine receptors associated with peripheral afferent nerve fibers, which then relay cytokine signals to brain regions (Liang et al.). TNF plays a major role in the propagation of inflammation due to the activation and recruitment of immune cells via the TNF receptor 1 (TNFR1) (Fischer and Maier).

$PM_{2.5}$ also activates NF-κB, a group of protein transcription factors regulating the expression of genes associated with inflammation (Ku et al.; National Cancer Institute). The activation of NF-κB downregulates the expression of microRNA-574-5p (miR-574-5p), which can increase the expression of the B-site amyloid precursor protein cleaving enzyme 1 (BACE1) enzyme (Ku et al.). The NLRP3 inflammasome has been presented as a possible mediator between $PM_{2.5}$ and systemic inflammation and the brain (Brugge et al.). NLRP3 inflammasome activation can produce sterile inflammation—inflammation without bacterial or viral infection.

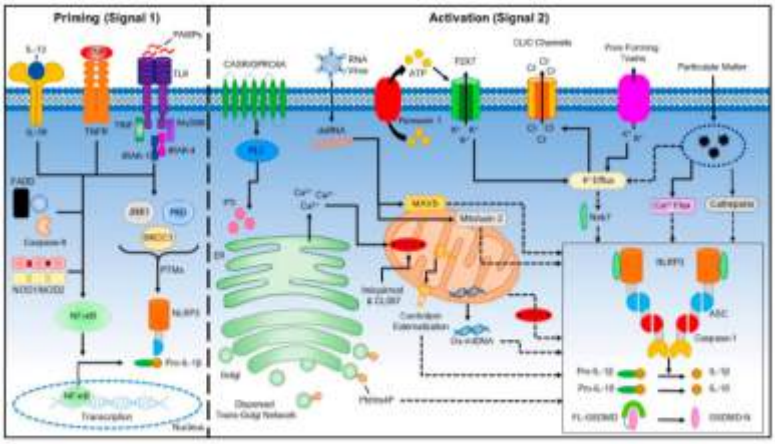


Figure 3: "Pathways for NLRP3 inflammasome activation, illustrating priming (left) and activation (right). The interaction of particulate matter with these pathways is included in the upper-right corner (reproduced from Kelley et al., 2019)." (Brugge et al.)

As seen in Figure 3, this can then produce IL-1β and IL-18. These interleukins can trigger a signaling cascade involving IL-6 and CRP in a positive feedback loop (Brugge et al.). These inflammatory biomarkers can then lead to low-level, chronic systemic inflammation, which is the underlying mechanism by which $PM_{2.5}$ influences neurological health (Brugge et al.). It is important to note that the NLRP3 inflammasome has not been confirmed to be a mediator—it has only been proposed considering existing evidence (Brugge et al.).

Neuroinflammation can lead to increased oxidative stress through the release of ROS and reactive nitrogen species (RNS) (Fischer and Maier). TNFR1 can directly lead to oxidative stress by activating the enzymes associated with the production of ROS and RNS (Fischer and Maier). “IL-1β, IL-6, and TNF-α reduce the synthesis of 5-HT (serotonin) by stimulating indoleamine 2,3-dioxygenase (IDO)," (Liang et al.). IDO can convert tryptophan into kynurenine, and the activation of the kynurenine pathway can result in the production of ROS and RNS (Liang et al.). Superoxide anion radicals (SAR), like the oxide ion, can act as a co-substrate of IDO, which may lead to the superinduction of the neurotoxic kynurenine pathway in the presence of excessive oxidative stress, reducing the survivability of neuronal cells (Liang et al.). This can further promote neuronal damage and inflammation, resulting in a feed-forward loop of neurodegeneration. (Fischer and Maier). ROS can also further promote the synthesis and release of inflammatory factors (Liang et al.).

Neuroinflammation and BDNF

Brain-derived neurotrophic factor (BDNF) plays a critical role in memory formation and hippocampal plasticity. The BDNF gene encodes BDNF mRNA transcripts, which then give rise to mature BDNF after translation and cleavage (Liang et al.). Mature isoforms of BDNF can bind to tyrosine kinase receptor B (TrkB), which can promote cell survival and enhance hippocampal long-term potentiation. After binding, several signaling pathways, including PI3K/Akt, MEK/ERK, and LKB1/AMPK, are activated. These can target the mammalian target of rapamycin complex-1 (mTORC1), a growth regulator (Liang et al.). The activation of mTORC1 can lead to increased synaptic formation, enhanced neural plasticity, and rapid antidepressant effects. The signaling pathway also regulates autophagy and promotes nerve regeneration, which regulates depression through the bidirectional control of autophagy and autophagic flux (Liang et al.). The AMPA receptor-BDNF-mTOR signal pathway is suggested to be crucial for enhancing synaptic function in the medial prefrontal cortex (Liang et al.). Inflammatory cytokines including IL-1β, IL-6, and TNF-α can inhibit the neuroprotective effects of BDNF. Levels of IL-1β higher than the physiological range can result in the upregulation of the p38 mitogen-activated protein kinase (MAPK), which can potentially disrupt BDNF-dependent synaptic plasticity processes (Liang et al.).

Impairment of the Neurovascular Unit, Mitochondria, and Endoplasmic Reticulum

The core of the neurovascular unit (NVU) is the “orchestrated effort of multiple cells engaged in distinct signaling pathways and effector systems across the entire cerebrovascular network,” (Calderón-Garcidueñas et al.). The NVU “is composed of neurons, glial cells, brain microvascular endothelial cells (BMECs), pericytes, and the extracellular matrix,” (Gong et al.). The NVU controls the permeability of the BBB and also protects the brain from blood-borne, endogenous, and exogenous substances (Gong et al.). In a study conducted among participants in the Metropolitan Mexico City (MMC) area who were exposed to high levels of particulate matter, every NVU component was compromised with exposure to NP (Calderón-Garcidueñas et

al.). Damage to the NVU can lead to abnormal ROS generation, mitochondrial dysfunction, and altered mitochondrial quality control (Calderón-Garcidueñas et al.)

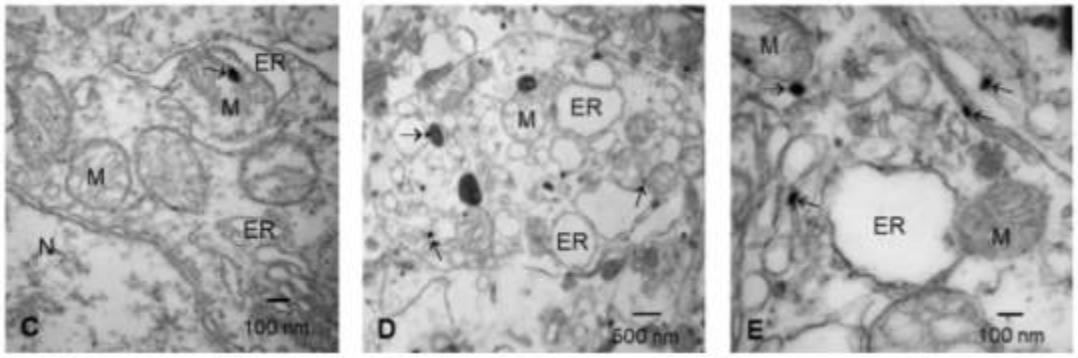


Figure 4: "Mitochondria with nanoparticles is a common finding in exposed subjects (C). There is also extensive dilatation of the endoplasmic reticulum ER (D) and the mitochondria-ER contact sites (MERCs) are abnormal (E)." (Calderón-Garcidueñas et al.)

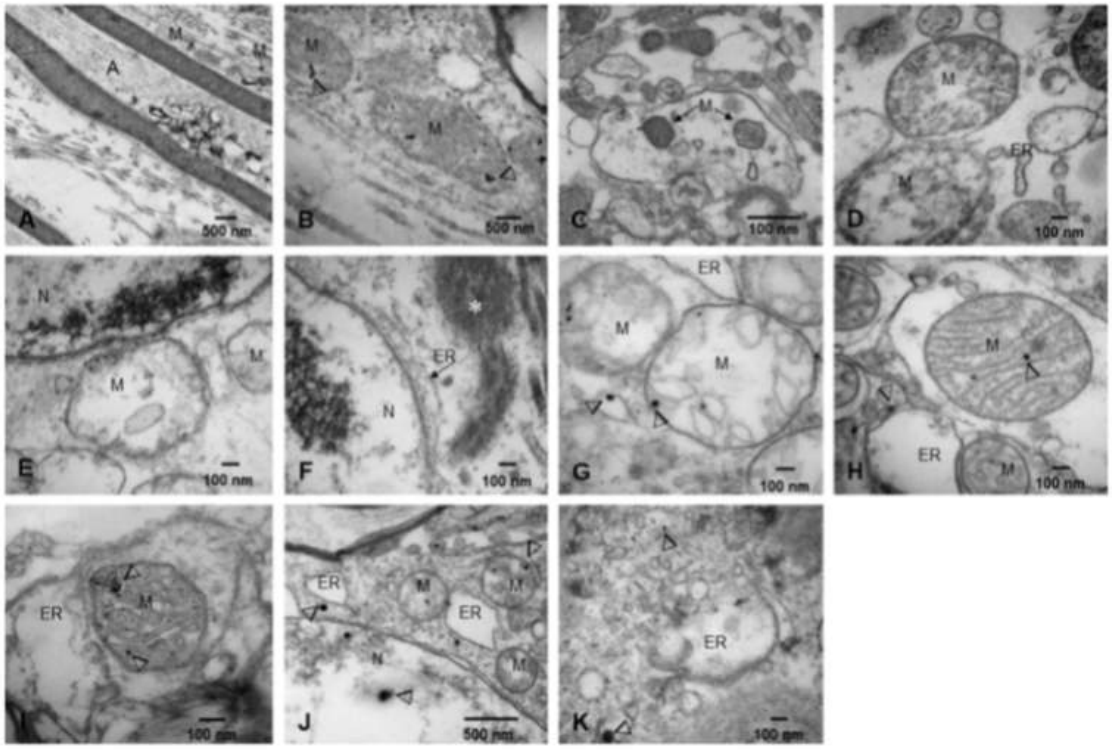


Figure 5: "Extensive mitochondrial and endoplasmic reticulum ER abnormalities are seen in neurons, axons and dendrites. Vagus nerves at the cervical level (A and B) show axonal vacuolated structures containing particulate matter and abnormal mitochondria with well-defined in situ nanoparticulate material (arrowheads). Dendrites contain abnormal mitochondria with short or absent cristae and abnormal matrix compartmentalization (C and D). Abnormal ER and presence of nanoparticles in mitochondria are the most common finding in exposed young subjects (E-J). In K, abnormal fragmented ER and nanoparticles of different sizes (arrowheads)." (Calderón-Garcidueñas et al.)

As seen in Figure 4, among exposed subjects in the MMC study, mitochondria with nanoparticles, extensive dilation of the endoplasmic reticulum (ER), and abnormal mitochondria-ER contact sites (MERCs) were found (Calderón-Garcidueñas et al.). MERC physical interactions are key for the exchange of calcium ions and lipids, the regulation of ATP generation, and mitochondrial division (Calderón-Garcidueñas et al.). As seen in Figure 5, there were several more abnormalities in the mitochondria and ER of subjects in the MMC study (Calderón-Garcidueñas et al.). In fact, this organelle damage was present in every single MMC resident tested and is associated with their exposure to UFPs (Calderón-Garcidueñas et al.).

The ER is "critical for the handling of misfolding and stress-damaged proteins in route to clearance by the ubiquitin-proteasome system (UPS)," (Calderón-Garcidueñas et al.). An intact ER is needed for the elimination of abnormal proteins. Consequently, ER alterations can lead to protein degradation failure and the aggregation of abnormal proteins such as H-tau and α-synuclein (Calderón-Garcidueñas et al.). NPs can also act as molecular chaperones to carry out protein folding, destabilization, and protein aggregates (Calderón-Garcidueñas et al.). An increased protein concentration and the destabilization of protein folded states may promote amyloid aggregates (Calderón-Garcidueñas et al.).

The mitochondria have the vital cellular functions of fission and fusion, axonal development and regeneration, ATP generation, buffering of systolic Ca, and the production of ROS (Calderón-Garcidueñas et al.). The expression of the Mfn-1 and Mfn-2 proteins mediates fusion (Salvi et al.). Fission protein DROP-1 is expressed as a result of heightened oxidative stress and facilitates degradation and cell death (Salvi et al.). The mitochondria produce ROS and electron transport chain (ETC) complexes 1 and 3, which produce the oxide ion during the electron transfer process. Inflammatory cytokines, including TNF-α, can stimulate ROS production in the mitochondria by inhibiting the ETC and altering membrane permeability. Furthermore, mitochondrial dysfunction in depression may lead to elevated ROS (Liang et al.). The mitochondria also regulate inflammation, immune responses, and mitophagy (Calderón-Garcidueñas et al.). Mitophagy is important in the brain because it will avoid the accumulation of damaged, ROS-producing mitochondria, which can trigger inflammasome activation. Mitochondria are subjected to high mutation rates because of a lack of protective histones. Damaged mitochondria may induce Toll-like receptors, formyl peptide receptors, inflammasomes, and the cGMP-AMP synthase cGAS/Stimulator of IFN genes pathway (Calderón-Garcidueñas et al.). Continuous exposure to toxic factors like particulate matter can "result in chromatin reorganization, autophagy, mitophagy, and apoptosis designed to get rid of unviable cells and damaged mitochondria," (Calderón-Garcidueñas et al.). Under extreme conditions, "sequestration of damaged mitochondria in double membrane vesicles and their delivery to lysosomes as a quality control pathway is no longer possible," (Calderón-Garcidueñas et al.). Exposure to pollution may override the defensive and protective measures in the mitochondria, including superoxide dismutase (SOD) expression, and the brain, including mitoquinone mesylate (Mito-Q), an antioxidant (Salvi et al.).

The brain and mitochondria are also at greater risk for oxidative stress since those regions already promote the accumulation of ROS (Salvi et al.). A buildup of ROS often leads to mitochondrial impairment, either through increased oxidative stress in the brain or mitochondrial oxidative stress (Salvi et al.). The hippocampus is a more significant site of mitochondrial damage than the amygdala. In a study, after exposure to particulate air pollution, the hippocampus showed increased expression of both the fusion and fission pathways, which suggests that mitochondrial impairment occurred (Salvi et al.). There was also increased oxidative stress in the prefrontal cortex (PFC), hippocampus, and amygdala, marked by an increased amount of 8-isoprostane, a biomarker for oxidative stress (Salvi et al.).

Oxidative Stress Can Increase BACE1 Expression

$PM_{2.5}$-induced oxidative stress can activate NF-κB (Ku et al.). NF-κB downregulates miR-574-5p through its activation and reverse modulation on miR-574-5p (Ku et al.). The dysregulation of miR-574-5p has been linked to neurodegenerative diseases, and a study involving mice has shown a statistical difference in miR-574-5p expression decrease at higher PM2.5 dosages (Ku et al.). miR-574-5p binds to the 3'UTR of the BACE1 enzyme, keeping it deactivated (Ku et al.). When miR-574-5p is downregulated, BACE1 expression increases. The BACE1 enzyme, along with γ-secretase, catalyzes the cleavage of Amyloid Precursor Protein (APP) in its amyloidogenic pathway (Ku et al.). This can lead to the production and accumulation of amyloid-beta (Aβ) peptides, which is a hallmark of neurodegenerative diseases, including Alzheimer's disease. BACE1 activity can also affect normal synaptic function, which is a hallmark of early cognitive impairment (Ku et al.).

The Link Between Oxidative Stress and Depression

As explained earlier, excessive ROS generation and lack of efficient antioxidants, characteristics of oxidative stress, may trigger stress responses, neuroinflammation, neurodegeneration/an imbalance of synaptic plasticity, tissue damage, and cell death (Correia et al.). These are all mechanisms underlying the development and progression of depression (Correia et al.).

Oxidative Stress and the HPA Axis

The hypothalamic-pituitary-adrenal (HPA) axis responds to physical or psychological stressors by releasing corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) through neurons in the paraventricular nucleus (PVN) and hippocampus (Correia et al.; Liang et al.). This hormone facilitates posttranslational cleavage of pro-opiomelanocortin mRNA (POMC) in the anterior pituitary gland, resulting in the synthesis and secretion of adrenocorticotrophin (ACTH) (Liang et al.). ACTH is then released into the circulatory system, stimulating the release of glucocorticoids (GR), mineralocorticoids (MR), and other players in the stress response, including catecholamines (Correia et al.; Liang et al.). The hippocampus is an important site where GR and MR receptors mediate negative feedback regulation and plays a

vital role in the end stage of the stress feedback process. MR receptors initiate the stress response while GR receptors terminate it (Liang et al.). In humans, the primary adrenal steroid is cortisol, while the primary adrenal steroid in rodents is corticosterone. HPA axis dysfunction and elevated CRH mRNA expression levels are reported in those suffering from depression (Correia et al.). Prolonged exposure to high glucocorticoid levels can result in synapse loss, neuronal death, and changes in neuronal dendrites (Correia et al.). High levels of stress are reportedly connected with disruptions in serotonergic-related pathways, the volume of some brain areas including the hippocampus and prefrontal cortex, and epigenetic changes in genes including BDNF (Correia et al.). Hyperactivation in the HPA axis may be induced by increased ROS production. Additionally, "glucocorticoids released in response to HPA axis activation induce activity of cellular reduction-oxidation systems," and "the activation of glucocorticoid receptors (GRs) in response to stress leads to an increase in mitochondrial membrane potential, calcium-holding capacity, and mitochondrial oxidation," (Correia et al.).

Several studies support the connection between HPA hyperactivity and oxidative stress. In vivo studies showed that after exposure to corticosterone, there was an increase in oxidative markers, including lipidic peroxidation, and a decrease in antioxidant enzymatic systems, including catalase (Correia et al.). In this study, oxidative injury in the hippocampus of rats led to impaired cognitive function (Correia et al.). Another study in mice showed that corticosterone and brain oxidative stress marker levels increased after the induction of depression and anxiety-like behaviors with pressure injury (Correia et al.). The administration of vitamin D3 to the mice counteracted depression-like behavior and oxidative stress triggered by repeated corticosterone administration. Lipid peroxidation, protein carbonyl, and nitrite levels decreased after treatment, leading to an improvement in depression symptoms (Correia et al.). In mice, lutein and the carotenoid crocin-I showed antidepressant-like activity (Correia et al.). Crocin-I induced the activity of antioxidants (Correia et al.). In another study, "catalpol administration to mice also inhibited HPA axis hyperactivity (reflected in lower levels of corticosterone, ACTH, and CRH), central inflammation, and oxidative damage via regulation of the NF-κB and Nrf2 pathways, producing antidepressant effects in mice injected with corticosterone," (Correia et al.). Antioxidant enzyme activity was also induced by Myrcia pubipetala Miq administration in mice treated with corticosterone (Correia et al.). L-cysteine was also found to be effective as an antidepressant on corticosterone-induced oxidative stress by reducing plasmatic corticosterone levels and increasing antioxidant defenses (Correia et al.). In vitro, the addition of Hericium erinaceus to rat pheochromocytoma cells (PC-12) led to neuroprotective effects (Correia et al.). Hericium erinaceus relieved oxidative stress caused by high corticosterone doses. This effect was shown by enhanced endogenous antioxidant enzyme activities, attenuated intracellular ROS levels, and protection from apoptosis triggered by high ROS levels (Correia et al.). Furthermore, in a study involving 60 nurses, it was found that as stress and anxiety levels increased, the amount of oxidant markers and cortisol levels increased (Correia et al.).

Oxidative Stress, Neuroinflammation, and Associated Depression

Depressed individuals may have elevated levels of immune markers, including granulocytes, monocytes, TNF, IL-6, and microglial activation (Correia et al.). Another study showed that mRNA levels of proinflammatory markers, including IL-1β, IL-6, TNF-α, and lymphotoxin and anti-inflammatory markers including IL-10 and IL-1 receptor antagonist (IL-1RA) were increased and decreased, respectively, in the prefrontal cortex of depressed individuals who committed suicide (Correia et al.). Furthermore, proinflammatory markers TNF-α and IL-1β may trigger an imbalance of the HPA axis by inhibiting the transcription of c-fos and AVP (Liang et al.). As explained before, oxidative stress is associated with increased neuroinflammation in the body (Brugge et al.; Wang et al.; Ku et al.; Fischer and Maier). High ROS production levels in mitochondria are also connected with inflammation and producing oxidative stress (Correia et al.). An overactivated inflammatory system and increased ROS levels act synergistically to promote the onset and development of depression (Correia et al.). This suggests that oxidative stress may be correlated with hyperactivation in the HPA axis, which can further lead to the onset of depression.

Several studies demonstrate this connection. In a study, oxidative stress from indoor air pollution from solid fuel use markedly increased inflammation and promoted depression and cognitive function impairment in middle-aged and older Chinese adults (Correia et al.). NOX1 is a source of ROS, and raftlin is important in inflammatory processes. A study in humans found that there was increased serum NOX1 and raftlin levels in depressed patients compared to controls (Correia et al.). Antidepressants such as imipramine were found to have reduced microglial activation, decreasing proinflammatory cytokine levels and reversing stress-induced social avoidance in mice (Correia et al.). A study revealed that resveratrol, a compound linked to antioxidant, anti-inflammatory, and antidepressant effects, attenuated depressive-like behavior in stressed animals. It also mediated alteration in the hippocampal levels of anti-inflammatory and proinflammatory cytokines and induced antioxidant actions (Correia et al.). Depressive symptoms in COVID-19 survivors were associated with high inflammation levels that also correlated with low glutathione levels in the anterior cingulate cortex (Correia et al.). In another study, administering the anti-inflammatory compound muscone ameliorated depression-like behavior through the regulation of inflammatory responses and the improvement of oxidative stress markers, particularly malondialdehyde (MDA), SOD, and GPx (Correia et al.). MDA may lead to protein damage and advanced lipoxidation product generation with proinflammatory characteristics, detected in patients with depression (Correia et al.). Administering apple phenolic extracts against lead acetate ($Pb(Ac)_2$)-induced cognitive impairment and depression/anxiety-like behavior in mice attenuated the increased cellular oxidative damage and the levels of proinflammatory cytokines IL-1β, IL-6, and TNF-α (Correia et al.). A similar study with cinnamic acid improved lipopolysaccharide (LPS)-induced depressive-like behaviors in mice through the inhibition of neuroinflammation and oxidative stress. The proinflammatory markers (IL-6 and TNF-α) and oxidative stress markers (SOD, glutathione, and MDA) were highly improved in the hippocampus and cortex of the depressed mice (Correia et al.). Curcumin was also found to attenuate LPS-induced anxiety/depression-like behavior in rats by decreasing

cerebral oxidative stress (Correia et al.). Curcumin increased SOD and GPx enzyme activity and also reduced MDA concentrations. Curcumin also exerted anti-inflammatory properties through the inhibition of microglial activation (Correia et al.). p-Coumaric acid has a protective role against inflammation and oxidative stress in various diseases and was found to reverse depression-like behavior by inhibiting advanced glycation end products (AGEs) and the receptor for AGE (RAGE) and AGE-RAGE-mediated neuroinflammation (Correia et al.). Administering quercetin to zebrafish alleviated oxidative stress and neuroinflammation induced by LPS, ameliorating behavioral abnormalities (Correia et al.). Versus LPS-treated fish, proinflammatory compounds, including TNF-α and IL-1β, decreased, and the antioxidant glutathione increased (Correia et al.). According to another study, "similar results were obtained with LQFM212, 2,6-di-tert-butyl-4-((4-(2-hydroxyethyl)piperazin-1-yl)methyl)phenol, a piperazine derivative," which "exhibited elevated antioxidant effects and also ameliorated LPS-induced behavioral, inflammatory, and oxidative changes in the tested animals," (Correia et al.). The flower essential oil of Tagetes minuta was found to attenuate oxidative stress and restore the cellular pathway BDNF-Akt/ERK2 (Correia et al.). This led to an attenuation in inflammation and depressive-like behavior in mice. It was found that astilbin, a compound with antioxidant characteristics, ameliorated depressive-like behavior through the regulation of astrocyte-mediated neuroinflammation caused by postnatal immune activation in mice (Correia et al.).

Oxidative Stress, Serotonin Imbalance, and Associated Depression

The monoamine hypothesis says that altered levels of monoamine neurotransmitters serotonin (5-HT), noradrenaline (NA), and dopamine (DA) in the CNS are associated with depression, with 5-HT being especially significant (Correia et al.; Liang et al.). The efficacy of SSRIs demonstrates that 5-HT may be highly involved in depression; however, the mechanisms are complex, and more research is still needed to understand these processes (Correia et al.). Oxidative stress can lead to many neurotoxic substances through the oxidation of the 5-HT precursor tryptophan. Oxidation-specific epitopes, including MDA, are known to have extremely proinflammatory properties and are correlated to depression through interactions with pathways including tryptophan/kynurenine (Correia et al.). Tryptophan can be metabolized to kynurenine through proinflammatory cytokines and directly through ROS, and "the molecule may be further metabolized to prooxidant compounds, 3-hydrocykynurenine and quinolic acid, associated with the pathogenesis of depression," (Correia et al.).

Several studies demonstrate these connections. Oxidative DNA and RNA damage was found to be attenuated after SSRI treatment in the case of unipolar depression (Correia et al.). A study in murine RAW264.7 macrophages showed that oxidative stress was reduced by 5-HT and its metabolites. 5-HT and its metabolites also prevented the production of inflammatory cytokines by macrophages (Correia et al.). Selenium-modified fluoxetine derivatives simultaneously target 5-HT reuptake (antidepressant action) and oxidative stress (Correia et al.). Mirtazapine and L-tryptophan, which are both associated with serotonergic-related pathways, have been found to counteract cellular stress induced by hydrogen peroxide; this underscores the

role of serotonergic pathways in oxidative stress (Correia et al.). Saffron extract has been found to protect human neurons from oxidative stress, stimulate the production of dopamine, 5-HT, and BDNF, and inhibit the expression of the 5-HT transporter (Correia et al.). In another study, "nacre extract from pearl oysters also suppressed LPS-induced depression and anxiety in mice," (Correia et al.). The high levels of oxidative stress induced by LPS were accompanied by changes in the 5-HT receptors 5-HT1A and 5-HT2A and BDNF, which suggests an antidepressant action by the nacre extract (Correia et al.). Administrating persimmon leaf extract, which is associated with antioxidant properties, to depressed mice "prevented dendritic spine loss through the inhibition of 5-HT reuptake, increasing 5-HT brain levels, alleviating the depressive-like behavior of the animals," (Correia et al.). Additionally, "in a postpartum depression rat model, supplementation with the probiotic Lactobacillus casei improved depression-like behaviors," (Correia et al.). The probiotic led to processes including enhanced expression of monoamines and the BDNF/ERK1/2 pathway and decreased levels of oxidative stress (Correia et al.).

Oxidative Stress, Synaptic Plasticity, and Associated Depression

As explained earlier, oxidative stress and related neuroinflammation can impair BDNF and synaptic plasticity through BACE1 regulation (Liang et al.; Ku et al.). Dysfunctional BDNF can lead to synaptic plasticity imbalance and decreased excitatory neurons and glutamate, promoting depression (Correia et al.). This is shown by decreased BDNF mRNA levels in postmortem depressed brains as a result of suicide. There was also increased BDNF expression in the hippocampus in patients treated with antidepressants (Correia et al.). Neurogenesis occurs in the lateral subventricular zone and dentate gyrus of the hippocampus in adults (Correia et al.). As explained before, oxidative stress mainly targets the hippocampus (Salvi et al.). Therefore, it may be suggested that oxidative stress may be correlated with impaired neurogenesis. This is further proved by the fact that corticosteroids reduce neurogenesis and BDNF, while 5-HT is known to enhance the process (Correia et al.). There was also an increase in neurogenic hippocampal markers after SSRI treatment (Correia et al.).

This connection is exemplified by several studies. Depression-like behavior was improved in mice through the administration of tilapia skin peptides, which regulated oxidative stress and neurogenesis (Correia et al.). The data suggest that "the BDNF/TRKB/CREB pathway may be involved in the antidepressant effects of these peptides, ameliorating neurogenesis and neural apoptosis," (Correia et al.). The compound also enhanced the Nrf2/HO-1 pathway, which regulates the expression of many antioxidant genes (Correia et al.). In a study, the BDNF-protein kinase B (Akt) / signal-related kinase 2 (ERK2) signaling pathway was restored after administering the flower essential oil of Tagetes minuta. Oxidative stress was also mitigated, leading to decreased depressive-like behavior in mice (Correia et al.). Melatonin was found to have "increased antioxidant markers and increased neurogenesis in the hippocampus and prefrontal cortex of rats previously treated methotrexate," (Correia et al.). Melatonin improved antioxidant defenses by improving the Nrf2 and BDNF expression, upregulating synaptic

plasticity, and enhancing doublecortin expression (Correia et al.). Doublecortin is an important marker of neurogenesis (Correia et al.). In another study, the antioxidant carvedilol was administered to depressed mice, and it increased brain glutathione and BDNF concentration while decreasing MDA levels, having antidepressant-like effects (Correia et al.). Luteolin-7-O-glucuronide also has reported antioxidant properties, and when administered to mice, improved depression-like behavior, activated BDNF signaling, and subsequently modulated neurogenesis and neuroplasticity (Correia et al.). In a study, rosmarinic acid "reversed LPS-induced depressive behaviors in mice by promoting the expression of the BDNF/Nrf2 pathway, also leading to the expression of antioxidant enzymes such as heme oxydase-1 and NAD(P)H quinone dehydrogenase 1 (NQO1), and downregulating the expression of proinflammatory genes," (Correia et al.). Depressive and anxiety behaviors in rats were alleviated by both celastrol and thymoquinone, which reverted the previously decreased concentration of acetylcholine, dopamine, and serotonin while increasing BDNF expression and downregulating oxidoinflammatory markers, including MDA and IL-6, in the rat brains (Correia et al.).

The Involvement of Menopause

Perimenopausal Depression

Perimenopausal depression is "characterized by the simultaneous occurrence of multiple menopausal symptoms that overlap with emotional disorders, like depression and no interest, sleep disorders, tiredness, low energy, no self-confidence, and thoughts of death," (Han et al.). Symptoms may vary from one culture or region to the next. Generally, women with higher education levels living in urban cities were more susceptible to menopause symptoms (Liang et al.). Menopausal depressive symptoms are the highest during the early perimenopause period, which means that this period is a better opportunity for effective treatment. However, the financial and healthcare burden for treatment is substantial (Liang et al.). Due to estrogen having neuroprotective effects, a decline in ovarian function and prolonged estrogen deprivation during menopausal transition may affect psychologic and cognitive well-being (Liang et al.). Menopausal transition and the perimenopausal stage are critical periods during which female patients may be susceptible to anxiety and depressive disorders (Liang et al.). Estradiol, a form of estrogen, increases 5-hydroxytryptamine receptor 2A ($5\text{-}HT_{2a}$) expression and diminishes serotonin catabolism by regulating tryptophan hydroxylase type 2 gene expression and a reduction in monoamine oxidase-A (MAO-A) levels and enzyme activity (Liang et al.). Decreased BDNF levels in the hippocampus and the PFC are associated with an ovariectomy, which led to depression due to estrogen deficiency in mice. Estrogen can increase BDNF expression and release through estrogen receptor β. The level of serum estradiol is significantly positively correlated with BDNF levels; conversely, a progressive reduction in estradiol in peripheral blood is associated with decreased BDNF serum quantity in perimenopausal women (Liang et al.). BDNF levels are negatively correlated with self-rating depression scores in women

with perimenopausal syndrome (Liang et al.). During perimenopause, there is a significant upregulation of pro-inflammatory cytokines secreted by CD4 T cells, including IL-8a and TNF-α. Estradiol levels in peripheral serum have an inverse relationship with serum IL-8 and TNF-α and microglial and astrocytic reactivity in perimenopausal women. Estrogen can inhibit the secretion of inflammatory factors by T cells and microglia, and in premenopause, β estradiol inhibits microglia-mediated astrocytic activation to alleviate neuroinflammation and the secretion of inflammatory mediators by microglia (Liang et al.). A decline in estrogen can lead to immune cell activation, which can then produce IL-1, IL-6, and TNF-α (Liang et al.). Estrogen can increase cGMP through the direct stimulation of granulosa guanylate cyclase and can also activate the NO/cGMP/PKG cascade reaction by increasing the synthesis of NO (Liang et al.). Decreased estrogen levels after menopause can lead to the decrease in cGMP levels in women's bodies (Liang et al.). Estrogen can regulate hypothalamic responses to stress by regulating CRH, oxytocin, and AVP gene expression in hypothalamic neurons and excitatory inputs to the PVN (Liang et al.). Perimenopausal estrogen deficiency leads to weakened regulation of the HPA axis response to external stress, and estrogen deficiency increases the sensitivity of the pituitary gland to CRH, thereby resulting in the overactivation of the HPA axis (Liang et al.).

Oxidative Stress Can Increase the Risk for Perimenopausal Depression

Estrogen normally reduces oxidative stress levels by regulating the synthesis of antioxidant enzymes in the body (Liang et al.). Through analysis of the derivatives of reactive oxygen metabolites (d-ROMs), a marker of oxidative stress, and the biological antioxidant potential (BAP), an indicator of protein levels, it was found that oxidative stress is already higher in postmenopausal women than premenopausal women (Liang et al.). It was also found that "malonaldehyde (MDA), 4-hydroxynenal (4-HNE), and oxidized lipoproteins (ox LDL) were higher in postmenopausal women than fertile women, while glutathione peroxidase levels were significantly higher in fertile women than in postmenopausal subjects, which indicated that the levels of oxidative stress in postmenopausal women increase while the synthesis of antioxidant enzymes decreases," (Liang et al.).

Furthermore, as was shown earlier, several of the mechanisms on which oxidative stress acts are already compromised in individuals undergoing perimenopause due to a lack of estrogen. Having these mechanisms be compromised by oxidative stress at the exact time they are already worsened during perimenopause may lead to a greater risk, ultimately leading to a greater risk of developing depression at the perimenopause stage.

As explained earlier, $PM_{2.5}$ exposure has several mechanisms by which it can cause or exacerbate oxidative stress in the body. Therefore, there may be a correlation between heightened $PM_{2.5}$ exposure and the incidence of depression in middle-aged women undergoing perimenopause.

Additional Exacerbating Factors

Along with being in a state of perimenopause, there are several other factors that may exacerbate the impacts of $PM_{2.5}$ and oxidative stress-mediated depression. The increased risk of MDD related to long-term $PM_{2.5}$ exposure was aggravated among people who have underlying chronic diseases, especially those with chronic inflammatory diseases, such as diabetes mellitus (Kim et al.). This risk was also heightened among those exposed to higher levels of $PM_{2.5}$, and it has been proposed that those with a relatively low income may also have a heightened risk (Kim et al.). Long-term $PM_{2.5}$ exposure may target the middle-aged and elderly demographic more and, especially in the elderly demographic, may lead to cognitive decline and neurodegenerative diseases, such as Alzheimer's disease (Kim et al.).

Existing Mitigation Techniques

There are several existing mitigation techniques in place to combat air pollution exposure and depression. Conventional antidepressant medications include tricyclic antidepressants (TCAs), including clomipramine, amitriptyline, and doxepin; monoamine oxidase inhibitors (MAOIs), including phenylethylhydrazine, brofaromine, toloxadone, and isohydrazine; and selective serotonin reuptake inhibitors (SSRIs), including fluoxetine, citalopram, escitalopram, and sertraline (Liang et al.). MAOIs and TCAs have been found to alleviate depressive symptoms by increasing the concentration of neurotransmitters like serotonin, norepinephrine, and dopamine. The design of SSRIs was predicated based on observations of lower levels of plasma tryptophan, a precursor to serotonin, and reduced serotonin levels found in the cerebrospinal fluid in patients with depression (Liang et al.). For perimenopausal depression, gonadal hormones like estrogen treatment are sometimes employed. These are aimed at slowing down the progression of depression and alleviating symptoms (Liang et al.). Estrogen has a variety of mechanisms contributing to the mitigation of perimenopausal depression. Estrogen facilitates the action of SSRIs, increasing the sensitivity of perimenopausal depression patients to SSRIs and subsequently improving treatment outcomes (Liang et al.). Estrogen replacement therapy is able to prevent and counteract modifications made through high oxidative stress by acting as a regulator of key antioxidant gene expression (Liang et al.). For example, the co-treatment of estradiol with glutamate can help alleviate neuroinflammation, neurodegeneration, and synapse loss. It does this by reducing brain oxidative species through upregulation of the Nrf2/HO-1 antioxidant pathway (Liang et al.). New pharmacotherapeutic agents like serotonin/norepinephrine reuptake inhibitors (SNRIs), glutamatergic agents (ketamine and d-cycloserine), and selective estrogen receptor modulators (SERMs) are gradually being used to treat perimenopausal depression due to having better efficacy and milder side effects (Liang et al.).

Along with treatments for depression and menopausal effects, other measures can be taken to mitigate air pollution exposure. General rules such as staying indoors during peak pollution times or using air purifiers can reduce total air pollution intake. Specifically, air purifiers with HEPA, high-efficiency particulate air, filters are designed to trap $PM_{2.5}$ (Saenz et al.). Indoor air may seem clean, but it may still have air pollution, and heating, ventilation, and

air conditioning (HVAC) systems without proper filtration may spread pollutants (Saenz et al.). Air purifiers with HEPA filters generally cost more than standard filters (Saenz et al.). This may pose an obstacle to obtaining air purifiers with HEPA filters for lower- and middle-income households, indirectly marginalizing them.

Along with air purifiers, communication through mediums such as infographics can inform the public on how to avoid excessive air pollution exposure. One study found that more than half the survey respondents indicated an intention to change behavior based on the infographic's recommendations about air pollution avoidance (Ginzburg et al.).

Proposed Mitigation Techniques

Several methods found to alleviate oxidative stress and related depression-like symptoms were discussed earlier (Correia et al.).

Additional Proposed Mitigation Techniques

Polyphenols, which are plant-derived natural compounds, have been found promising for being therapeutic against inflammation-associated depression due to their strong antioxidant properties (Correia et al.). Vitamin E, an antioxidant compound, has also been reported to be beneficial for oxidative stress and inflammation in depressive patients (Correia et al.). Upregulation of the mitochondrial antioxidant sirtuin 3 was able to rescue low levels of neurogenesis in stress-induced depressed mice, and the compound has been deemed to be a promising strategy to improve depressed behavior (Correia et al.). Substances upregulating BDNF were shown to mitigate the effects of chronic stress-induced depression by ameliorating the HPA axis and hippocampal GR dysfunctions and the 5-HT system (Liang et al.). The overexpression of miR-574-5p in the hippocampal region can be a possible solution, since it was shown to decrease BACE1 expression, restore synaptic function, and improve spatial memory and learning (Ku et al.). BACE1 expression was also silenced by stereotaxically injecting a lentivirus vector (LV) expressing BACE1 shRNA in the hippocampal area (Ku et al.). NF-κB inhibitor SC-514 and NF-κB p65 shRNA silencing techniques were able to diminish miR-574-5p repression, but $PM_{2.5}$-induced increase in BACE1 levels was not fully rescued. In a study, the Mito-Q antioxidant was able to reduce the adverse effects of oxidative stress but was not equally effective in all brain regions and thus was not enough to completely mitigate oxidative stress and its effects (Salvi et al.). While these mechanisms have been tested in lab procedures and studies, it is important to note that many have only been tested in rodents and not humans. Furthermore, these tests have often been used to prove the connection between oxidative stress and its related mechanisms rather than being tested to procure a treatment. Therefore, these tests cannot be directly labeled as treatments without further research being conducted.

Conclusion

For the relationship between $PM_{2.5}$ exposure, oxidative stress, and depression to be confirmed to be causal, more research needs to be done in these areas. Considering the high rates

of depression in recent years, along with persistent high air pollution levels, it is imperative that research be conducted in this area to inform research about treatment. Furthermore, the more evidence that is available to back the diverse harms of air pollution, the higher the chances are at changes in policy and law concerning emissions.

This problem marginalizes low-income and minority communities (Calderón-Garcidueñas et al.). More work should be done to reduce the disparity between groups facing this problem. Problems such as the high relative costs of air purifiers with HEPA filters may contribute to these disparities in health safety (Saenz et al.).

Specifically, more research should be devoted to studying the mechanisms by which oxidative stress may lead to depression. This includes more research concerning HPA axis hyperactivation, neuroinflammation, serotonin imbalance, and synaptic plasticity imbalance. Furthermore, more research should be conducted on the role of perimenopause in the effectiveness of oxidative stress in inducing depression, since women at this stage are at higher risk due to a lack of estrogen (Liang et al.). More research is also needed to confirm the efficacy of several tests as possible treatments, as that would allow for a variety of treatments to be implemented to mitigate depression, considering that therapy resistance is common for this condition (Correia et al.).

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Likes, Dopamine, and Dependency: The Neurological Basis of Social Media Addiction in Adolescents By Sheen Kaul

Abstract

Social media use amongst adolescents has increased dramatically over the past decade, alongside rising rates of anxiety, depression, and compulsive use behaviors (Twenge et al., "Increases in Depressive Symptoms"; Valkenburg et al.). While much public discussion focuses on social and psychological factors, less attention has been placed on the brain-based factors that contribute to dependency. This paper examines how the adolescent brain's still-developing prefrontal cortex and heightened dopamine reward sensitivity create a distinctive vulnerability to social media addiction. This review draws on scientific studies to explore how unpredictable rewards, the need for social approval, and app features exploit adolescent brain responses (De et al.; Özüçetin). The findings suggest that compulsive social media use functions similarly to a behavioral addiction rooted in how the brain works. This has important implications for adolescent mental health, the guidance parents can provide, and the need for stronger regulations on social media platforms (Kuss and Griffiths; Henzel and Håkansson).

Introduction

The average teenager currently spends more than six hours daily on social media, texting, and other online activities (Anderson and Jiang; Anderson et al.). The question arises whether compulsive use is merely a matter of poor self-control or if underlying neurological mechanisms are at play. Over the past two decades, social media has become integral to adolescent life, with daily usage rates among 12th graders increasing sharply since the late 2000s (Twenge et al., "Trends in US Adolescents' Media Use"). As usage has grown, so too have concerns about adolescent mental health, with researchers, parents, and policymakers expressing alarm over rising rates of anxiety, depression, and sleep disruption among young people (Twenge et al., "Increases in Depressive Symptoms"; Gupta et al.). Public discourse often centers on behavioral explanations, such as excessive screen time, insufficient parental monitoring, and social pressures from online comparison (Strasburger et al.; Dwivedi et al.).

While these factors contribute, they frequently overlook the neurological processes that drive such behaviors. Adolescence represents not only a social or emotional transition but also a period of significant neurological development, rendering the brain particularly susceptible to reward-driven compulsion in ways that differ from adults (Blakemore; Crone and Konijn). This paper contends that the adolescent brain's still-maturing dopamine reward system, in conjunction with the engagement-oriented design of social media platforms, creates measurable neurological conditions that increase the likelihood of dependency (De et al.; Perales et al.). Framing this issue solely as a matter of willpower misinterprets the scientific evidence. This review examines prefrontal cortex development, dopamine reward circuitry, and neuroimaging evidence of platform-induced brain activation to argue that adolescent social media dependency is

fundamentally a neurological issue rather than a purely behavioral one (Wadsley and Ihssen; Özüçetin).

Methodology

This narrative literature review examines the neurological processes underlying adolescent social media dependency. The objective was to synthesize findings from neuroscience, psychology, psychiatry, and adolescent development to identify consistent patterns across these disciplines. A systematic search was conducted using Google Scholar, PubMed, PsycINFO, and JSTOR, employing search terms such as adolescent social media use, social media addiction, dopamine reward system, prefrontal cortex, brain development, social media mental health, and behavioral addiction. Boolean operators were applied as appropriate. Titles and abstracts were initially screened for relevance, followed by a full-text review based on inclusion and exclusion criteria.

Only studies directly addressing adolescent brain development, dopamine signaling, or social media dependency were included in the final review (Valkenburg et al.; Crone and Konijn; Wadsley and Ihssen). Systematic reviews, meta-analyses, longitudinal studies, and neuroimaging research were prioritized, as these designs generally yield stronger evidence than single cross-sectional studies (Marciano et al.; Kuss and Griffiths). Findings from various disciplines were then compared to identify recurring themes and areas of substantive disagreement. Studies reporting contradictory findings were also included to provide a balanced evaluation of the current evidence.

Literature Review

Adolescent vulnerability to social media dependency is not simply a behavioral issue or a matter of parenting. Research across neuroscience, developmental psychology, and addiction studies shows that the teenage brain has biological characteristics that make it especially prone to compulsive social media use. At the same time, platforms are designed to take advantage of those characteristics. This literature review examines five main areas: adolescent brain development, the dopamine reward system, social media as a neurological trigger, conflicting evidence and alternative perspectives, and the ongoing debate over whether problematic use should be considered a clinical addiction.

The Adolescent Brain

To understand social media dependency, it is first necessary to understand how the adolescent brain develops. The human brain does not fully mature until around the mid-twenties, with the prefrontal cortex being the last region to do so (Blakemore). The prefrontal cortex handles impulse control, planning, and the ability to think through consequences. In adolescents, it remains structurally and functionally incomplete. MRI studies confirm that major changes in brain structure and function continue throughout the teenage years, showing that the systems

responsible for self-regulation are still very much in development (Wadsley and Ihssen; Crone and Konijn).

At the same time, the limbic system, which controls emotions and rewards, is highly active during this period. Research shows that teenagers are especially sensitive to social acceptance and rejection, which makes their responses to social media interactions more intense than those of adults (Blakemore; Özüçetin). Neuroplasticity strengthens this effect further: younger brains adapt and rewire more easily in response to experience, meaning that repeated exposure to dopamine-triggering platforms can leave lasting changes in reward pathways (Elsayary and Ragab). The patterns that form during adolescence often carry into adulthood (Crone and Konijn). Taken together, this evidence shows that adolescents are neurologically more prone to compulsive behavior, and that expecting them to regulate their social media use without support is both unrealistic and unfair (Valkenburg et al.; Gupta et al.).

The Dopamine Reward System

Dopamine is often called the brain's pleasure chemical, but that description misses something important: dopamine is mainly a chemical of anticipation, not satisfaction (Perales et al.). It rises in response to the expectation of a reward, and it rises most when that reward is unpredictable. This is why opening a social media app can feel exciting even when nothing interesting appears; the brain has already responded in anticipation (De et al.). The ventral tegmental area and nucleus accumbens are central to this process. These regions do not only respond to meaningful stimuli but also to the simple possibility of reward, in ways that are like how the brain responds to addictive substances (Özüçetin; Wadsley and Ihssen).

Social media platforms are built around a variable reward schedule, where rewards arrive unpredictably, a pattern that research consistently shows is more effective at sustaining engagement than any predictable reward structure (Kuss and Griffiths; Henzel and Håkansson). For adolescents, whose brains are already more sensitive to dopaminergic signals, this effect is especially strong (Crone and Konijn).

Over time, compulsive use can produce a neurological shift where users crave the activity but feel less satisfied by it, a pattern well documented in internet gaming disorder research, where sensitivity to natural rewards decreases as sensitivity to digital interactions increases (Peng and Liao; Perales et al.).

Social Media as a Dopamine Delivery System

Experimental research confirms that social media platforms directly activate these neurobiological responses. fMRI studies have shown that posts with higher like counts significantly activate brain regions associated with social evaluation and reward, regardless of the content itself (Özüçetin; Wadsley and Ihssen). Teenagers with stronger neural responses to social approval tend to be heavier social media users, which supports a cycle where use increases reward sensitivity, and greater reward sensitivity encourages even more use (De et al.; Elsayary and Ragab). Emotional effects are equally important. Negative online interactions such as

criticism, exclusion, or receiving fewer likes can activate the same brain regions involved in processing physical pain (Sinha et al.).

This means the pain of being left out on social media is not simply emotional; it is neurologically real. Meanwhile, design features like infinite scroll and pull-to-refresh are not accidental choices. They are deliberately built to take advantage of the brain's reward circuitry and remove the natural stopping points that might otherwise interrupt the cycle (Kuss and Griffiths; De et al.). The neurological effects can even become structural: research has found reductions in grey matter and other physical changes in the brains of people with problematic internet use, suggesting that the effects of dependency go well beyond behavior (Elsayary and Ragab; Wadsley and Ihssen). The interconnected neurological mechanisms discussed in this section are summarized conceptually in Fig. 1.

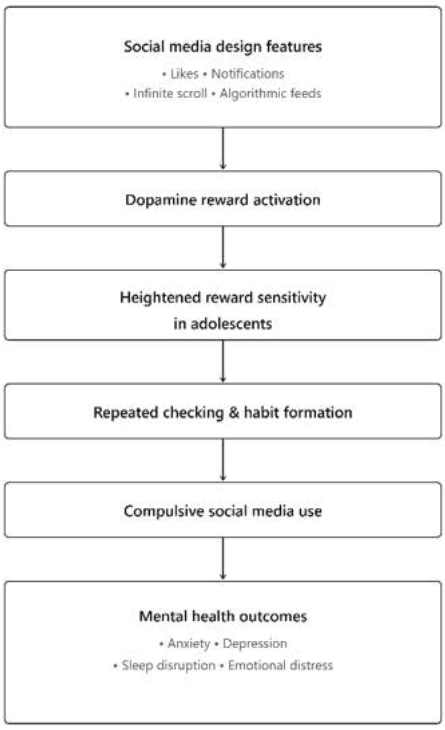


Fig. 1. Conceptual model illustrates how social media design features activate dopamine-mediated reward pathways, reinforce habitual checking, and contribute to compulsive use and adverse mental health outcomes in adolescents

The Addiction Debate

Whether adolescent social media use qualifies as a clinical addiction remains genuinely debated (Valkenburg et al.; Henzel and Håkansson). The classification matters because it affects how the problem is treated and who is considered responsible. Platforms have a clear interest in keeping that question unresolved, since formal recognition of social media as an addiction would

strengthen the case for regulation. The criteria most commonly used to assess addiction include salience, tolerance, mood modification, withdrawal, loss of control, and conflict (Peng and Liao). Research has found meaningful links between heavy social media use and these symptoms (Kuss and Griffiths). Younger adolescents are consistently shown to be at greater risk for problematic use, which is tied to higher rates of depression and anxiety (Twenge et al., "Increases in Depressive Symptoms"; Fisher et al.), a risk that grows when teens use social media to cope with or escape negative emotions (Henzel and Håkansson; Bekalu et al.).

Not all researchers agree this rises to the level of addiction, though: some caution against applying addiction criteria developed for substance use disorders to behavioral patterns without stronger evidence that the same mechanisms are at work (Marciano et al.). The distinction matters, because compulsivity driven by choice is different from compulsivity that leaves no real alternative (Perales et al.). Even so, the absence of a formal diagnosis does not make the harm any less real.

A significant minority of adolescents develop dependency patterns consistent with behavioral addiction (Peng and Liao; Henzel and Håkansson), while a larger group experiences serious psychological effects that fall short of a clinical diagnosis (Valkenburg et al.). Given what is now known about adolescent brain development and the deliberate design of these platforms, it is becoming increasingly difficult to argue that these outcomes are simply a matter of personal willpower (De et al.; Kuss and Griffiths).

Conflicting Evidence and Alternative Perspectives

The mechanisms described throughout this paper, prefrontal immaturity, dopamine-driven compulsion, and platform designs built to exploit these vulnerabilities, have meaningful empirical support. However, significant debate remains over whether social media dependency should be formally recognized as a true behavioral addiction, and several lines of research complicate the picture presented so far. The most fundamental disagreement concerns classification. The DSM-5 recognizes only gambling disorder as a formal behavioral addiction, with internet gaming disorder listed merely as a condition requiring further study, and social media dependency appears nowhere in it (Henzel and Håkansson). Kuss and Griffiths caution that applying addiction criteria to social networking risks pathologizing behavior that is simply generationally normal; being constantly connected has become the default way teenagers communicate and relate to one another, which makes it genuinely difficult to separate a disorder from a cultural shift.

They raise an important distinction as well: people may not be attached to platforms themselves but to the underlying need a platform satisfies, connection, validation, or entertainment, meaning two adolescents with identical usage patterns could be having completely different psychological experiences. Henzel and Håkansson found that even among the heaviest users in their sample, most did not score above clinical cutoffs for problematic use, suggesting that high frequency alone does not define the condition. Others caution more broadly that current evidence is not yet sufficient to justify formal diagnostic classification, and that

labelling a near-universal behavior as addiction risks overstating what the data actually shows (Marciano et al.; Dwivedi et al.).

A related problem concerns how dopamine is discussed in this context. The phrase "social media releases dopamine" appears frequently, but it oversimplifies how the reward system actually works.

Dopamine functions primarily in reward prediction and anticipation, not pleasure itself, and it responds most powerfully when rewards are uncertain rather than guaranteed (Perales et al.). What the evidence does support is that variable, unpredictable rewards like likes and notifications engage reward-learning circuitry in ways that fixed rewards do not, and repeated activation of that system may gradually reinforce compulsive checking behavior (Özüçetin; Kuss and Griffiths). Dopamine activity during social media use is evidence that reward circuitry is engaged. It is not, on its own, proof of addiction.

Many studies in this area also establish correlation without establishing cause. Henzel and Håkansson state explicitly that causality remains unstudied, and that it is equally plausible that adolescents already experiencing anxiety or depression turn to social media more rather than developing those difficulties because of it. Bekalu and colleagues raise the same concern, noting that emotional dependence on social media may reflect a pre-existing mental health condition rather than produce one. Marciano and colleagues found that when they isolated only longitudinal studies across their 78-study meta-analysis, general social media use did not significantly predict well-being over time at all, and only problematic use showed a modest negative effect. Longitudinal designs strengthen causal arguments, but they cannot eliminate every confounding variable, and factors like family conflict, poor sleep, or pre-existing vulnerability may be driving both the social media use and the distress simultaneously (Crone and Konijn).

The harm narrative also does not apply equally to every adolescent or every type of use. Dwivedi and colleagues identify meaningful benefits of social media: overcoming geographic barriers, maintaining friendships, enabling educational communities, supporting creative expression, and amplifying voices that traditional media excludes. Bekalu and colleagues found that when social media use was measured as ordinary routine integration rather than emotional dependence, it was positively associated with social well-being, positive mental health, and self-rated health. Marciano and colleagues found that active use, posting, communicating, and sharing, was consistently linked to small positive effects on well-being across dozens of studies, while passive scrolling showed no meaningful relationship in either direction. Individual differences shape outcomes too: personality traits, pre-existing conditions like ADHD, and factors such as impulse control and self-esteem all determine who is most vulnerable, meaning two teenagers spending identical time online may experience it very differently (Kuss and Griffiths; Crone and Konijn).

Finally, the research base itself has real limitations. Self-reported screen time is a notoriously inaccurate measurement tool, and prevalence estimates for problematic use range from under 2% to over 30% depending on how the construct is defined and measured (Henzel

and Håkansson; Marciano et al.). Kuss and Griffiths identified at least six competing psychometric scales in the literature, each built on different theoretical frameworks with different cutoff scores, making direct cross-study comparison unreliable. Many neuroimaging studies involve small sample sizes that limit generalisability (Wadsley and Ihssen), and most behavioral studies are cross-sectional rather than longitudinal (Valkenburg et al.). As Marciano and colleagues point out, algorithmic feeds and infinite scroll did not exist when many foundational addiction scales were designed, meaning some instruments may be measuring behaviors that no longer reflect how adolescents engage with these platforms.

The neurological case for adolescent vulnerability is real and growing, but it is not yet complete, and methodologically consistent, large-scale, longitudinal research remains the field's most pressing need.

Discussion

The evidence reviewed throughout this paper (adolescent brain development, dopamine-mediated reward processing, and deliberate platform design) describes a set of connected neurological and psychological mechanisms that together drive problematic social media use (De et al.; Özüçetin; Perales et al.). Taken together, these findings support the idea that social media can function similarly to a behavioral addiction (Kuss and Griffiths; Henzel and Håkansson). Although these findings build a compelling case, substantial debate remains over whether social media should be formally classified as one (Valkenburg et al.; Marciano et al.). The central question is whether social media addiction represents a true behavioral addiction comparable to gambling disorder, or a pattern of problematic use that has not yet met the scientific threshold for formal recognition (Peng and Liao). Rather than reflecting several independent processes, the evidence points to a single interconnected neurological explanation.

The prefrontal cortex, which handles impulse control, planning, and long-term decision-making, does not fully mature until the mid-twenties (Blakemore). The brain's reward circuitry, by contrast, develops much earlier, creating a developmental gap between seeking rewards and regulating behavior that is especially wide during adolescence (Crone and Konijn). Dopamine responds most strongly to unpredictable rewards rather than guaranteed ones (Perales et al.). A notification that might contain a like or comment therefore produces a stronger anticipatory response than one that always delivers the same result, which reflects the same variable reinforcement principle seen in gambling (Kuss and Griffiths; Özüçetin). Modern social media platforms incorporate features such as infinite scroll, variable notifications, algorithmically curated content, and visible engagement metrics that are built around these reward-learning principles, making sustained engagement more likely (De et al.; Sinha et al.). Together, these neurological vulnerabilities and platform features create a feedback loop in which persuasive design repeatedly activates reward circuitry at exactly the stage of development when adolescents have the least capacity for inhibitory control (Elsayary and Ragab; Crone and Konijn).

A consistent pattern emerges across multiple types of research. Neuroimaging studies regularly report increased activation in reward-related brain regions during social media use, particularly when adolescents receive likes or social validation (Wadsley and Ihssen; Özüçetin). Behavioral studies link heavier social media use to weaker impulse control, increased social comparison, and greater emotional distress (Twenge et al., "Increases in Depressive Symptoms"; Fisher et al.; Gupta et al.). Longitudinal studies, though fewer in number, suggest these effects may accumulate over time rather than remain isolated experiences (Twenge et al., "Increases in Depressive Symptoms"; Twenge et al., "Trends in US Adolescents' Media Use"). The convergence of findings across neuroimaging, behavioral, and longitudinal research strengthens the overall case, and consistent results across independent lines of research strengthens confidence in the overall conclusions (Valkenburg et al.; Marciano et al.).

Despite this growing body of evidence, the classification debate remains unresolved. Established behavioral addictions like gambling disorder are defined by symptoms including preoccupation, tolerance, withdrawal, loss of control, and continued use despite negative consequences. Some researchers argue that problematic social media use mirrors several of these features, pointing to compulsive checking, cravings for social validation, and emotional distress when access is restricted (Peng and Liao; Henzel and Håkansson). Others argue that labeling a near-universal behavior as addiction risks pathologizing ordinary technology use, and that current evidence is not sufficient to justify formal diagnostic classification (Marciano et al.; Dwivedi et al.). This review does not attempt to settle that debate.

What it does show is that the neurological mechanisms underlying problematic social media use are measurable and consistently observed, whatever terminology is ultimately used (Özüçetin; Wadsley and Ihssen). Several limitations should be kept in mind when reading this literature. Many neuroimaging studies involve small sample sizes, which limits how broadly their findings can be applied (Wadsley and Ihssen). Most research is correlational rather than experimental, so causal relationships cannot yet be confirmed with certainty (Valkenburg et al.). It remains unclear whether heavy social media use contributes to neurological changes, or whether adolescents with pre-existing vulnerabilities are simply more likely to use platforms heavily (Crone and Konijn; Naslund et al.). Many studies also rely on self-reported screen time, which tends to be inaccurate (Anderson and Jiang), and researchers use different tools and definitions of problematic use, making direct comparisons between studies difficult (Kuss and Griffiths; Peng and Liao).

As a literature review, this paper is shaped by the strengths and weaknesses of the existing research it draws on. Even without agreement on classification, the practical implications are significant. Parents should recognize that excessive social media use reflects more than a lack of self-discipline, as adolescents are engaging with technologies built around reinforcement-learning principles during a developmental period when executive control is still maturing (Blakemore; De et al.). Educators can strengthen digital literacy by teaching students not just to limit screen time but to understand how algorithms, notifications, and engagement features shape behavior (Moreno et al.; Strasburger et al.). Mental health professionals may

benefit from incorporating assessments of problematic social media use into routine adolescent screenings, regardless of whether formal diagnostic recognition follows (Naslund et al.; Bekalu et al.).

Policymakers should increasingly treat adolescent social media use as a public health concern that warrants evidence-based regulation, transparency, and continued research investment (Valkenburg et al.; Kuss and Griffiths). Whether or not problematic social media use is eventually classified as a behavioral addiction, the evidence reviewed in this paper makes one thing clear: adolescents are engaging with digital environments designed to exploit fundamental characteristics of the developing brain (De et al.; Elsayary and Ragab). Important questions about causality and classification remain unanswered, but the consistency of findings across neuroscience, psychology, and behavioral research highlights how urgent continued investigation is (Valkenburg et al.; Özüçetin). As platforms grow more personalized through artificial intelligence and more sophisticated recommendation systems, understanding their effects on adolescent brain development will only become more important for researchers, educators, clinicians, and policymakers alike (Crone and Konijn; Twenge et al., "Trends in US Adolescents' Media Use").

Future Directions

Current research has made real progress in connecting adolescent social media use to dopamine-driven reward processing and addiction-like behavior, but important questions remain (Valkenburg et al.; Kuss and Griffiths). Answering them will determine whether problematic social media use deserves formal recognition as a behavioral addiction, and what can be done about it. The biggest methodological problem is that most existing studies are cross-sectional, meaning they capture one moment in time rather than tracking change over years (Valkenburg et al.). That makes it impossible to know whether heavy social media use causes addiction-like symptoms, or whether already-vulnerable adolescents simply gravitate toward it more.
Long-term longitudinal studies are needed to establish which direction that relationship actually runs (Twenge et al., "Trends in US Adolescents' Media Use"; Crone and Konijn).

Neuroimaging research also needs to expand. The fMRI studies that exist tend to have small sample sizes and inconsistent findings (Wadsley and Ihssen). Larger studies that track prefrontal cortex development alongside social media use, and that account for individual differences in dopamine sensitivity, would give researchers a much clearer picture of why some adolescents develop compulsive patterns while others don't (Blakemore; Crone and Konijn).

A third gap is platform design itself. Researchers have not yet isolated which specific platform features drive compulsive use most strongly.
Infinite scrolling, notification timing, variable like-counts, and recommendation algorithms all function as potential reinforcement mechanisms, but their individual effects on adolescent neurology remain understudied (De et al.; Kuss and Griffiths). Naslund et al. note that future research must carefully consider the mechanisms involved in online peer-to-peer connections that may be fundamental in sustaining engagement, yet the neurological consequences of those

design choices, especially for developing brains, have rarely been examined in controlled settings.

Finally, research needs to move beyond documenting the problem toward testing solutions. Interventions like digital literacy education, school-based screen-time programs, family strategies, CBT, and platform-level policy changes all show theoretical promise, but the evidence base for what works is thin (Naslund et al.; Strasburger et al.). Building that evidence base should be a clear priority (Moreno et al.).

As social media evolves faster than the research studying it, the need for this work becomes more urgent. Interdisciplinary collaboration across neuroscience, psychology, and public health is the most realistic path toward both understanding social media dependency and protecting the adolescents most at risk from it (Valkenburg et al.; Özüçetin).

Conclusion

This paper sets out to answer one central question: why are adolescents so uniquely vulnerable to social media dependency? The answer is straightforward. Teens are more vulnerable because their brains are still developing, their dopamine systems are highly sensitive to unpredictable rewards, and social media platforms are designed to take advantage of both. The implications reach further than a research paper. For parents, this should reframe the conversation entirely.

A teenager who cannot stop scrolling does not lack discipline; their brain is responding to something it was never equipped to resist. For mental health practitioners, generic advice about screen time is no longer sufficient; what is needed are approaches that take the neurological reality seriously. For policymakers, science is developed enough to act on. Platforms that are deliberately designed to maximize engagement in developing brains should be held accountable for the consequences of that choice. There are still gaps in the research.

Causality is hard to establish, sample sizes in imaging studies are often small, and there is no single agreed standard for measuring addiction across studies. But these are arguments for moving faster, not for waiting. Until the conversation around adolescent social media use shifts from behavioral correction to neurological protection, society will continue asking developing brains to resist technologies deliberately designed to keep them engaged.

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The Effect of Median Household Income on HCAHPS Star Ratings in Metro Atlanta Hospitals By Gavin Cohen

Abstract

Patient satisfaction scores, measured by the Hospital Consumer Assessment of Healthcare Providers and Systems (HCAHPS) survey, are used by the Centers for Medicare and Medicaid Services (CMS) to indirectly influence hospital funding through the Value-Based Purchasing Program, yet community-level socioeconomic factors remain an understudied predictor of these scores [1]. This study examines whether median household income of a hospital's ZIP code is associated with HCAHPS summary star ratings across 23 different metro Atlanta hospitals. Income data was obtained from the U.S. Census Bureau American Community Survey (2024) and was matched with HCAHPS star ratings from the MCS Provider Data Catalog (July 2024 - June 2025). A moderate positive correlation was found ($r = 0.49$, $R^2 = 0.24$), with hospitals in lower-income ZIP codes averaging 2.58 stars compared to 3.0 stars in higher-income ZIP codes. Hospital system affiliation also independently influenced ratings, with Northside hospitals averaging 3.4 stars and Piedmont hospitals averaging 2.4 stars. These findings suggest that socioeconomic context should be accounted for when using HCAHPS scores to evaluate hospitals, as failure to do so may disadvantage hospitals serving lower-income communities.

Introduction

Patient satisfaction is increasingly important to how hospitals are evaluated and funded in the United States. The Hospital Consumer Assessment of Healthcare Providers and Systems (HCAHPS) survey, developed by the Centers for Medicare and Medicaid Services (CMS) and the Agency for Healthcare Research and Quality, measures patients' perceptions of their hospital experience across multiple categories, including nurse and doctor communication, responsiveness of staff, and overall hospital rating [2]. CMS uses these scores to indirectly influence hospital payments through the Value-Based Purchasing (VBP) program, making HCAHPS scores directly tied to hospital revenue and financial performance [1].

While patient-level predictors of HCAHPS scores such as age, education, and self-reported health status have been studied, community-level socioeconomic factors remain understudied. One exception is a study by Shulman et al. (2018) [1], which examined the effect of socioeconomic status on HCAHPS scores among 15,789 orthopedic patients at a single academic medical center. Using ZIP code median household income as a proxy for socioeconomic status, the study found that socioeconomic status independently affected HCAHPS scores even after controlling for factors already accounted for in CMS scoring. Notably, patients from lower-income ZIP codes rated hospitals higher than those from wealthier areas, a finding that contrasts with the hypothesis of the study and highlights the complexity of this relationship.

Given the limited research on community-level socioeconomic predictors of HCAHPS scores, and the absence of Atlanta-specific data, this study aims to examine whether median

household income of a hospital's ZIP code is associated with HCAHPS summary star ratings across 23 metro Atlanta hospitals. Understanding this relationship has consequences for how satisfaction scores are used to evaluate and fund hospitals, particularly those serving lower-income communities.

Methods

This study used a cross-sectional design to examine the association between median household income and publicly reported patient satisfaction scores among hospitals in the metro Atlanta area.

Patient satisfaction data was obtained from the CMS HCAHPS dataset, accessed through the CMS Provider Data Catalog [2]. The overall summary star rating, reflecting patient satisfaction on a 1 to 5 star scale, was used as a measure of patient satisfaction for the period July 2024 through June 2025.

Median household income data was obtained from the U.S. Census Bureau 5-year estimates (2024) at the ZIP code level, accessed through Census Reporter [3]. For two hospitals whose ZIP codes lacked the sufficient residential population for Census reporting (Grady Memorial Hospital, with the ZIP code 30303, and Emory University Hospital with the ZIP code 30322), county-level median household income for Fulton and DeKalb counties, respectively were used as a proxy.

Hospitals were included if they were located in one of the 11 core metro Atlanta counties (Fulton, DeKalb, Gwinnett, Cobb, Clayton, Cherokee, Forsyth, Henry, Rockdale, Douglas, and Fayette) and had a reportable HCAHPS summary star rating for the study period. Hospitals without a reportable star rating, including pediatric hospitals, were excluded. A total of 23 hospitals met the inclusion criteria.

Analysis was conducted in Google Sheets and included a Pearson correlation coefficient to assess the association between ZIP code median household income and HCAHPS star rating, an income threshold comparison dividing hospitals into those below and at or above $100,000 median household income, and a hospital system comparison grouping hospitals by parent health system.

Results

A total of 23 metro Atlanta hospitals were included in the analysis. Of these, 7 hospitals (30.4%) received a 2-star rating, 14 hospitals (60.9%) received a 3-star rating, and 2 hospitals (8.7%) received a 4-star rating. No hospitals received a 1-star or 5-star rating. Median household income across hospital ZIP codes ranged from $52,209 to $152,564, with a mean of $95,405. Star ratings were based on a combined total of 27,373 completed HCAHPS surveys, with an average survey response rate of 15.6% across all 23 hospitals.

A Pearson correlation coefficient was calculated to assess the relationship between median household income and HCAHPS star ratings. A moderate positive correlation was found ($r = 0.49$, $R^2 = 0.24$), indicating that as median household income of a hospital's ZIP increased,

patient satisfaction scores tended to increase as well (Figure 1). Median household income accounted for approximately 24% of variance in HCAHPS star ratings.

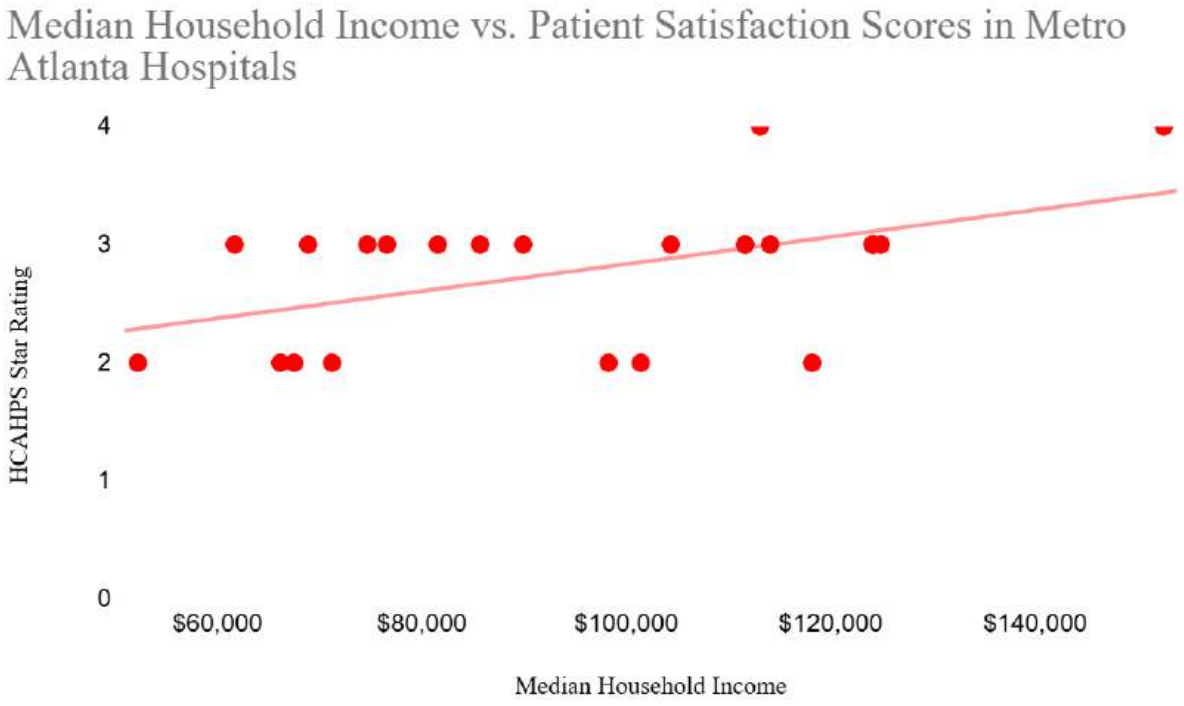


Figure 1

When hospitals were divided into two groups based on income, those in ZIP codes with median household incomes below $100,000 (n=12) averaged 2.58 stars, while those at or above $100,000 (n=11) averaged 3.0 stars. This difference of 0.42 stars is consistent with the overall positive correlation found between income and patient satisfaction.

A notable feature of the dataset was the concentration of hospitals at the 3-star rating. With 60.9% of hospitals receiving exactly 3 stars, most variation between hospitals was concentrated between the 2 and 3-star categories rather than spread across the full 5-star scale.

When grouped by parent health system, Northside hospitals had the highest average star rating (3.4 stars, n=5), then Emory (3.0 stars, n=5), Wellstar (2.5 stars, n=4), other or standalone hospitals (2.5 stars, n=4), and Piedmont (2.4 stars, n=5). Notable outliers included Wellstar North Fulton Medical Center, located in one of the higher-income ZIP codes at $118,170, which received only 2 stars, and Emory Hillandale Hospital, located in a lower-income ZIP code at $61,684, which received 3 stars.

Discussion

This study found a moderate positive correlation of r = 0.49 between median household income and HCAHPS star rating across 23 different metro Atlanta hospitals, supporting the

hypothesis that hospitals located in higher-income ZIP codes tend to receive higher patient satisfaction scores

However, this finding contrasts with Shulman et al. (2018), which found that patients from lower-income ZIP codes rated hospitals higher than those from wealthier ZIP codes when controlling for other factors at a single medical center. One possible explanation for this discrepancy is that Shulman et al. examined individual patient-level data at a single orthopedic center, while this study examined hospital-level star ratings across different hospitals in a single area. These different approaches may capture different aspects of the income-satisfaction relationship.

The finding that hospital system affiliation independently influenced satisfaction score is also noteworthy. Northside hospitals averaged 3.4 stars while Piedmont hospitals averaged 2.4 stars, despite both systems operating across a range of income levels. This suggests that factors beyond community income, like staffing, management practices, and resources, can play a meaningful role in shaping patient satisfaction.

The outlier findings are also important, as Wellstar North Fulton Medical Center, located in one of the highest-income ZIP codes in the dataset at $118,170, received only 2 stars, which is well below what the overall trend would predict. This suggests that income alone does not guarantee higher satisfaction scores, and that hospital-specific factors may matter more than community income effects. Also, Emory Hillandale Hospital performed better than expected given its lower-income ZIP code of $61,684, showing that the relationship between income and satisfaction is complex.

Limitations

This study has several limitations that should be considered when interpreting the findings. First, the sample of 23 hospitals is below the 30 or more pairs typically recommended for reliable correlation analysis, meaning these findings should be considered preliminary rather than conclusive.

Second, ZIP code median household income is not a perfect proxy for a hospital's true service area, as patients frequently travel from outside a hospital's immediate ZIP code, meaning that the income of that ZIP code may not accurately reflect the socioeconomic characteristics of the full patient population served.

Third, for two hospitals whose ZIP codes lacked sufficient residential population for Census reporting, county-level median household income was used instead. Fulton County income was used for Grady Memorial Hospital and DeKalb County income was used for Emory University Hospital. County-level income averages across a much larger area and may not accurately represent the income of patients these hospitals serve.

Fourth, the average HCAHPS survey response rate across hospitals in this study was 15.6%, below the national average of around 20-30%. This may introduce nonresponse bias as patients with stronger opinions would be more likely to respond to the survey with lower or higher numbers and not every person’s experience would be captured.

Finally, this study was limited to 11 core metro Atlanta counties, which may not fully represent the full metro Atlanta area, and could be expanded on in future studies.

Future Research

Future studies should aim to address the limitations in this analysis. Expanding the sample size to include hospitals across all 29 metro Atlanta counties, or applying a similar methodology to other U.S. cities, could make the study more generalized. Reaching or exceeding the recommended 30 hospital pairs could also strengthen the reliability of correlation findings.

Future research should also incorporate additional community-level variables beyond median household income, like poverty rate, race, insurance type distribution, and education level. Including these variables would allow researchers to better isolate the independent effect of income on patient satisfaction while controlling for other socioeconomic factors.

A more precise measure of a hospital's service area would also improve the accuracy of the income proxy. Finally, examining HCAHPS scores across multiple years rather than a single period would allow researchers to assess whether the relationship between income and patient satisfaction is consistent over time or shifts from policy changes or economic conditions.

Conclusion

The study found a moderate positive correlation between median household income and HCAHPS patient satisfaction star ratings across 23 different metro Atlanta hospitals, supporting the hypothesis that hospitals in wealthier communities tend to receive higher satisfaction scores. However, income alone does not fully explain the variation in ratings, as there is a concentration of scores at 3 stars, notable outliers, and differences in average ratings across hospital systems that suggest that other factors, like management practices, staffing, and communication, play an important role

These findings raise important questions about how HCAHPS scores are used to evaluate and fund hospitals. If satisfaction scores are partly shaped by the socioeconomic characteristics of a hospital's surrounding community instead of the actual care received, hospitals serving lower-income communities may be at a disadvantage in value-based payment systems. As CMS continues to reimburse hospitals based on patient satisfaction scores, policymakers should consider whether socioeconomic adjustment factors are needed to ensure hospitals serving lower-income populations are evaluated fairly.

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The Role of Consistent Exercise in Improving Mobility and Preventing Osteoporosis
By Ashmi Patel

Abstract

Osteoporosis isn't something most people think about until it becomes a problem, usually after a bad fall or a sudden fracture. By then, the disease has already been slowly weakening bones for years and with people living longer and spending more time sitting than generations before us, this issue is showing up in larger numbers and at earlier ages. Keeping the body moving well is becoming more important than ever.

This paper takes a look at how different kinds of exercise help protect our bone structure. Activities that place stress on the bones, like lifting weights or jogging, allow the body to reinforce and rebuild those particular bone tissues from the stress they undergo. Additionally, mobility work and stretching help make movement safer by reducing stiffness and lowering the chance of falling, even though they don't build bone directly. These activities not only help build or maintain bone density but also allow people to move confidently as they age, reducing their chances of falling and sustaining fractures. The goal of this review is to show that everyday exercise, not medication alone, can lower osteoporosis risk and support independent living throughout adulthood.

Introduction

Osteoporosis is a progressive skeletal condition caused by diminished bone density and degeneration of the bone's internal structure; both lead to an increased probability of suffering from a fragility fracture (Lane, 2006). Osteoporosis tends to develop gradually and can be asymptomatic, although a fracture will commonly occur in areas of the body where bones are likely to break, e.g., hips, vertebrae, and wrists, at some point during the disease process (NIH, 2021). There is no specific background characteristic for developing osteoporosis; however, postmenopausal women bear the brunt of the disease because they experience sudden hormonal shifts leading to significantly greater rates of bone resorption compared to bone formation (StatPearls, 2023). It is estimated that there are between 200-500 million people worldwide who have osteoporosis and that approximately one-third of the world's women over the age of 50 and one-fifth of the world's men will suffer an osteoporotic fracture in their lifetime (WHO, 2022).

In addition to its skeletal implications, the impact of osteoporosis extends to long-term issues related to the individual's mobility, autonomy and overall quality of life. Functioning mobility-the capacity to walk, carry items, maintain balance and perform basic movement patterns-is fundamental to an individual's continued autonomy throughout his/her lifetime. As humans grow older, bone remodeling efficiency decreases, and bones are being destroyed faster than new bone is forming, ultimately causing a progressive decline in skeletal strength (Nature Reviews Rheumatology, 2024). If an individual has limited functioning mobility due to a lack of strength, pain or fear of falling, disuse leads to further loss of bone and muscle mass, creating a self-perpetuating cycle that further increases the risk of a fracture, as well as long-term morbidity and potentially, mortality (JAMA, 2023). In the United States, osteoporosis results in more than

two million fractures each year, many resulting in long-term disabilities or loss of independence (U.S. Bone Health Alliance, 2020).

Instead of waiting until a person experiences a problem and then treating them reactively, preventing osteoporosis and maintaining functional mobility requires proactive actions. Physical activity is the most effective, inexpensive and scalable non-pharmacological intervention there is in order to maintain the strength of the skeletal system. Activities that involve weight bearing and high impact are effective in stimulating osteoblastic activity and bone mineralization, whereas resistance training increases muscle force, and subsequently, the load placed on bones, thus increasing the structural strength of bones (Harvard Health, 2021). Mobility and balance training provide complementary benefits by increasing joint range of motion and neuromuscular control, therefore decreasing the chances of falls and subsequent fractures (CDC STEADI, 2022).

Background: Understanding Bone Physiology

How Bones Grow & Remodel

Osteoblasts form and produce new bone tissue called osteoid and regulate minerals primarily calcium phosphate and harden it (Zhao et al., 2021). They are specialized bone forming cells derived from mesenchymal stem cells, responsible for synthesizing the osteoid (organic bone matrix) and coordinating its mineralization with calcium phosphate (Zhao et al., 2021). This is to build and repair bone tissue. They are crucial for skeletal development, growth, and the process of bone remodeling. They work in concert with bone resorbing osteoclasts to maintain bone density and strength (Zhao et al., 2021).

Osteoclasts are large, multinucleated cells that act as the "demolition crews," breaking down and reabsorbing old or damaged bone tissue (Zhao et al., 2021). They secrete acids and enzymes into specific areas on the bone surface, creating small pits or cavities, and then absorb the broken-down material, which allows the body to reuse the minerals and makes room for new bone (Zhao et al., 2021).

Bone remodeling is the lifelong process in which old bone is removed by osteoclasts and new bone is replaced by osteoblasts (Allen & Burr, 2014). This occurs in tiny areas across the skeleton called BMUs (basic multicellular units). It occurs in a tightly coordinated way, crucial for maintaining bone strength, repairing microdamage, and regulating mineral (calcium/phosphorous) balance (Allen & Burr, 2014). This adaptation process allows bones to respond to mechanical stress as supported by Wolff’s law, ensuring structural integrity while also serving mechanical needs (WebMD, 2023).

Humans reach peak bone mass between the 20s to early 30s, with significant accrual happening during the rapid growth of childhood and adolescence, specifically puberty (Smith et al., 2024). The exact timing varies by skeletal site and individual. It generally plateaus in late teens/early twenties in females and mid-twenties in males (Smith et al., 2024). Bone density holds steady until around age 50, when gradual loss begins.

Bones weaken with age because the balance between bone resorption and formation shifts: the rate of bone removed by osteoclasts exceeds the rate of new bone formation by osteoblasts (Nature Rheumatology, 2024). This leads to thinning bone, bigger marrow spaces, increased porosity, and changes in collagen. It makes bones less dense, structurally weaker, and prone to fractures. This process is accelerated by inflammation and reduced levels of growth factors like IGF-1 (Nature Rheumatology, 2024).

In women especially, testosterone declines significantly after menopause (Premenopausal women have 15–70 ng/dL and postmenopausal have 5–25 ng/dL) (Norton et al., 2023). Testosterone is key in building muscle mass because it boosts muscle protein synthesis—the process of repairing muscle fibers after exercise, making them stronger and bigger—and weakened muscles lead to osteoporosis (Norton et al., 2023).

Risk Factors for Osteoporosis

Women are at a higher risk of osteoporosis primarily because the rapid decline in estrogen levels during menopause causes an imbalance in bone remodeling (Frontiers in Physiology, 2022). Bone is lost a lot faster than it can be built, and this process is accelerated in the years immediately following menopause (StatPearls, 2023). Estrogen plays a crucial protective role in maintaining bone health; it inhibits bone resorption, promotes bone formation, and modulates inflammation (Frontiers in Physiology, 2022).

Genetics are a major determinant of skeletal strength and peak bone mass (Endocrine Reviews, 1999). Maximum bone density is achieved in early adulthood. Age is the strongest single risk factor for osteoporotic fracture (Lane, 2006). With aging, the natural bone remodeling cycle shifts, leading to an imbalance where more bone is resorbed than formed (Nature Reviews Rheumatology, 2024).

Nutrition is crucial for bone health at all life stages, playing a critical role in prevention and management. Low intake of essential nutrients like calcium, vitamin D, and protein contributes to lower bone mineral density, early bone loss, and increased fracture risk (Endocrine Reviews, 1999). Menopause accelerates bone loss due to sharp declines in estrogen, disrupting the bone remodeling balance by boosting osteoclasts and suppressing osteoblasts (PMC, 2023). This leads to bone breakdown more than formation, causing rapid density loss, especially in the first years after menopause (Frontiers in Physiology, 2022). Estrogen normally slows osteoclast activity and encourages osteoblast differentiation; once production declines, this process does as well (PMC, 2023).

Mobility: What It Is and Why It Matters

Mobility is people's ability to move around their environment covering physical aspects related to health and aging (Applied Mobility Review, 2020). Ability to move the body and get around is studied for balance, gait, strength, and its connection to aging, disability, and quality of life (PMC, 2020). Exercise benefits mobility because it increases joint range of motion and strengthens muscles (Mayo Clinic, 2022).

Mobility differs from flexibility and strength because mobility is active control through range of motion, while flexibility is passive movement (PMC, 2021). Mobility combines flexibility with strength, stability, and coordination to move well. Strength is the ability to generate force, providing power and stability, but lacks the controlled movement component of mobility. Strength training can improve ROM. Flexibility is how far a muscle can stretch (passive); mobility is how well one can control that stretch; strength allows for stability and usage of control (PMC, 2021).

Mobility declines as people age due to slower gait, reduced muscular strength, balance issues, and stiffer joints (JAMA Network Open, 2023). These changes create increased fall risk, slower walking speed, and higher energy cost for movement, impacting independence (JAMA Network Open, 2023). Interventions like exercise, sensory rehabilitation, and accessible environments can help maintain function (PMC, 2016). Key changes include muscle loss, joint stiffness, sensory deficits, and altered gait patterns (PMC, 2016).

Limited mobility increases fracture risk by causing bone weakening (disuse osteoporosis), reducing muscle strength, and impairing balance (JAMA Network Open, 2023). This leads to more falls, the primary cause of fractures in older adults with osteoporosis (JAMA Network Open, 2023). This creates a cycle: fractures limit movement, weaker bones and muscles increase fall risk, raising the likelihood of future fractures (Arthritis Foundation, 2022).

How Exercise Improves Bone Density

Weight-Bearing Exercises

Some activities that help bones support body weight are walking, running, dancing, hiking, stair climbing, sports like tennis, and strength training with weights or bands (Harvard Health, 2021). These activities force bones to work against gravity, stimulating them to get stronger and increase density. Swimming and cycling are excluded because support is external (Harvard Health, 2021).

Walking, running, and jumping stimulate bone growth by creating mechanical stress (PMC, 2020). Higher-impact activities are more effective for increasing bone density than walking alone (PMC, 2020). Brisk walking helps maintain bone mass. Jumping enhances trabecular bone by thickening it, while running increases trabecular number, improving bone structure (PMC, 2020).

Impact forces stimulate osteoblasts through mechanotransduction—physical stress converted into biochemical signals promoting bone formation (PMC, 2016). Osteocytes act as the primary mechanosensors detecting stress (PMC, 2016). Wolff's Law explains bones adapt to mechanical load, growing stronger with stress and weakening with inactivity (WebMD, 2023).

Resistance Training & Strength Training

Lifting weights creates mechanical stress and tension on bones, signaling osteoblasts to increase density (Mayo Clinic, 2023). This follows Wolff's law, where bone adapts to loads and becomes stronger (WebMD, 2023).

At the cellular level, resistance training causes microscopic muscle fiber damage that activates satellite cells, increasing protein synthesis and hypertrophy (NIH, 2022). This process involves growth factors such as IGF-1, testosterone, and growth hormone (NIH, 2022).

Muscles pulling on bone generate forces sensed by osteocytes, triggering remodeling—called mechanotransduction—and strengthening bone to prevent osteoporosis (CDC Bone Health, 2022).

Flexibility & Mobility Training

Stretching increases the extensibility of musculotendinous units, allowing wider joint motion (Mayo Clinic, 2022). Reduced stiffness enhances mechanical joint function. Consistent stretching reorganizes connective tissues for smoother movement (Mayo Clinic, 2022).

Improved mobility helps the body adjust its center of gravity when walking on uneven terrain. Flexible hips and ankles allow larger "recovery steps" after slips, preventing falls (Harvard Health, 2021). Dynamic mobility routines such as leg swings, torso twists, and hip circles help functional movement, while yoga and Tai Chi combine flexibility with control (NIA, 2021).

Balance & Neuromuscular Training

Even minor falls can cause fractures in individuals with osteoporosis due to weakened bone microarchitecture (Bone Health & Osteoporosis Foundation, 2021). Preventing falls is the most effective way to avoid fractures (CDC STEADI, 2022).

Balance training strengthens core and lower limb muscles while improving neural stability pathways (CDC STEADI, 2022). Enhancing postural sway control helps the body stay upright during challenging tasks (Arthritis Foundation, 2022).

Proprioception improves through one-leg stance drills, foam pads, tandem walking, and Tai Chi, forcing the brain to process joint and muscle feedback more effectively (Arthritis Foundation, 2022).

Exercise & Bone Health Through the Lifespan

Childhood & Adolescence

Adolescence is the most critical time for bone development because ~90% of peak bone mass is acquired by age 18 in girls and 20 in boys (NIH Bone Health, 2021). During puberty, the skeleton responds strongly to mechanical loading (AAOS, 2022). Failing to maximize bone density increases adult risk (NIH Bone Health, 2021). Exercise stress stimulates bone formation and raises peak bone mass (AAOS, 2022).

Adulthood

Exercise in adulthood maintains bone density and slows age-related bone loss (AAOS, 2022). Weight-bearing and resistance exercise keep osteoblasts active. After age 40, the goal shifts to preserving the "bone bank" accumulated earlier (AAOS, 2022). Combining

weight-bearing exercise, muscle strengthening, and balance training helps maintain density and prevent falls (AAOS, 2022).

Menopause & Older Adulthood

Estrogen suppresses osteoclast activity, protecting bone (Endocrine Society, 2023). The drop in estrogen during menopause removes this protection, causing bone resorption to outpace bone formation (Endocrine Society, 2023).

Resistance training becomes essential because mechanical loading stimulates osteoblast activity even with diminished hormones (NIA, 2021). sarcopenia reduction improves balance and spine protection. Mobility maintains ankle/hip ROM and reduces fall risk by widening “base of support” (Harvard Health, 2021).

Conclusion

Exercise serves as the most powerful non-pharmacological tool for building bone density and maintaining skeletal strength throughout the lifespan. While weight-bearing activities and resistance training build the "bone bank" during youth, consistent mobility and balance training provide the critical defense needed to prevent injury-causing falls in later adulthood. By prioritizing these physical habits early, individuals can drastically improve their long-term health outcomes and mitigate the risks associated with osteoporosis. Ultimately, the transition from sedentary lifestyles to consistent, technology-supported movement routines is essential for a future of healthy, fracture-free aging.

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Measuring the Emotional Toll of Patient Care in Regards to Its Contribution for Burnout
By Fahed Ahmed Badreddine

Abstract

In today's society, burnout throughout the healthcare industry has increased astronomically. Cardiology, in particular, has become one of the most prominent fields necessary to study. The numbers alone in cardiology have led to the overall stigma and attention viewed throughout burnout in the healthcare industry. This study sought to uncover the emotional toll of patient care in regard to its contributions to burnout amongst cardiologists. Through the utilization of a Delphi study, iterative consensus was carefully developed amongst the participants, which in turn helped provide clear statistically significant results. Across all three rounds, several solidified results evidently emerged based on participants' overall agreement. A serendipity was indisputably in favor. Although the outcome of this study was to measure the aspect of emotional toll with relation to patient care, the most prominent results stated that factors other than emotional toll in respect to patient care were the leading causes of burnout. Such contributors that were Patently identified were administrative burden and long work load and hours. Inclusively, the findings of this study demonstrated clear divergence in respect to the original research question and goal assigned for this research paper, thus providing notional, novel, and clear contributions for the cardiology community.

Introduction and Literature Review

Introduction

A rising and ongoing systemic problem in healthcare today is the prevalent recurrence of burnout. Burnout is increasingly recognized as a serious, worldwide public health concern, and in the healthcare field, “over half of attending physicians report experiencing symptoms of burnout” (Reith). Throughout all of the specialties in the healthcare field, recent trends of burnout in cardiology have led to a proliferation of studies that show compelling results. In a study with over 2000 cardiologists, symptoms of burnout were reported by approximately 1 in 4 respondents, equating to 26.8% (Alexandrou et al.).

The purpose of this study is to evaluate whether the emotional tool associated with patient care is a primary factor of burnout or if there are other underlying characteristics that may emerge. Although burnout may often be the most frequently discussed problem, there remain several things under burnout that accumulate and lead to its formation. As Mehta and Murphy put it, “The advancements in technology, advent of electronic health records, and rising legislative regulations place a tremendous burden on healthcare workers, negatively affect well-being and work–life integration, and contribute to high burnout rates in medicine.”

When assessing the characteristics that accumulate towards burnout, two of the most prominent factors are administrative burdens and electronic health records. As Alexandrou et al. put it, “The factors contributing most to burnout among cardiologists include bureaucracy and

excessive administrative workload." In addition, the other major factor of burnout stems from administrative burdens. Administrative burdens themselves do not directly lead to burnout; rather, the subcategory under administrative burdens leads to the formation of that category and hence the problem, burnout. Some negative components that fall under the category of administrative burdens are poor work-life integration and a high volume of tasks that are beyond direct patient care, which in turn leads to the formation of burnout (Mehta and Murphy).

Literature Review

Another associated characteristic with burnout is long work hours. On average, cardiologists work around 57 hours a week and have an average of around 106 hours on call in a month (Ross et al.). In a study assessing cardiology trainees in the United Kingdom, 76% of cardiologists reported long hours and workloads, with a correlation of burnout (Jenner et al.). An often overlooked component of burnout arises from poor prognosis. In fact, physicians experiencing such levels of burnout are more likely to make major medical errors and patient risk, which entails increased patient mortality and poorer outcomes (Reith).
Interpersonal challenges, particularly difficult interactions with patients' families, constitute another important factor of burnout. In cardiologists day-to-day practices, the additional component of patients' families being difficult provides yet another crucial aspect of burnout. In a study done by Rubin et al., cardiologists who were exposed to high emotional situations, such as complex and difficult interactions with distressed families during severe end-of-life or critical cardiac events, experienced a significant increase in burnout.

One of the toughest challenges for all researchers in this domain is gathering novel and different responses amongst cardiologists. Currently, research on burnout is only identified through physician studies in which single surveys are being used and only one specialty in cardiology (McCammon et al.). This clearly reveals a gap as current studies rarely examine burnout through multiple subspecialties and through the usage of multiple rounds. To mitigate this, I used an innovative approach called a Delphi study. I gathered several different types of cardiologists, such as electrophysiologists, interventional cardiologists, non-invasive advanced heart failure cardiologists, and general cardiologists. Furthermore, my study consisted of three rounds with different open-ended response questions and deemed answers as justifiable through overall consensus agreement.

To address these gaps, the research question emerged: To what extent does the emotional toll of patient care contribute to burnout among physicians in the field of cardiology? Through current studies stating that burnout was the result of emotional toll towards patient care (Reith), I asserted my hypothesis that the highest emotional toll would come from excessive patient interaction and complex patient cases, which, hence, would significantly increase the risk of burnout among cardiologists based on prior research. Nevertheless, from the results of my medical study, the emotional toll of patient care did contribute to burnout; however, it was administrative burdens and high workloads that were the forefront reason for burnout. This

statistically significant study resulted in a novel design for assessing current burnout amongst cardiologists in the United States.

Methods

This study examined the extent of burnout of cardiologists from the emotional toll of patient care.The research question posited was: “To what extent does the emotional toll of patient care contribute to burnout among physicians in the field of cardiology?” Using the Create approach provided the most efficient way to answer this question, as the Delphi study fits right under this approach category, it could analyze participants' overall consensus and identify recurring themes in their answers.

The creative approach served as a necessary first step in providing new and unique insights among different specialties in cardiology. Many studies have done their research through the use of ongoing rounds rather than one open survey round and have focused on one type of specialty in cardiology. For example, one of the most predominant survey studies and analyses out there comes from the Journal of Invasive Cardiology. The title “Burnout in cardiology: a narrative review” and the leading author, Michaella Alexandrou, MD, provide a presentation and analysis of numerous different burnout studies pertaining to cardiology. However, a significant limitation is that they did not survey multiple different specialties among cardiologists or administer multiple rounds of questions. The doctors state that they used “one survey” and only focused on “cardiac imaging specialists” and, in another, “attending interventional cardiologists,” which, in turn, hinders the diversity of cardiology's many specialties and subspecialties and provides multiple rounds of questions, which impacts results when assessing burnout's implications. Similarly, research by Panagioti et al. failed to identify more than one subset group of cardiologists, sticking only to “general cardiologists,” and stated that their survey was sent only “once” to the cardiologists. To address this gap that was seen amongst numerous different studies and analytical articles, this study focused on 2 factors that almost all other studies failed to account for and implement. Multiple rounds of assessing burnout through questionnaires and having numerous different specialties and subspecialties from the field of cardiology, rather than one group of cardiologists. These two were synthesized together by the use of a Delphi study and were deemed the most practical solution for answering this research question.

Variables and Subjects/Selection Criteria

Two variables were tracked. First, emotional toll (the mental and emotional cost of dealing with patients and stressful situations). Secondly, physician burnout. This is defined as a state of long-term stress where a doctor feels mentally, physically, and psychologically unable to perform their everyday activities like before. These variables played a crucial role in providing context for the problem. The summation of two sources proved to be crucial for identifying these and other variables, including “electronic health records (EHRs),” stated by Michaella Alexandrou, MD, and “excessive workload,” by Laxmi S. Mehta, MD. For participant selection, the inclusion and exclusion criteria for subject identification were simple. Participants had to be

actively licensed practicing cardiologists in a hospital. Inactive cardiologists and other types of physicians were excluded from the potential subject participants. This criterion helped filter out and minimize the outliers and extraneous variables when analyzing the data from the participants. Two studies proved beneficial in determining and defining their study populations. The study done by Sharma et al. provides a large, diverse study with male and female cardiologists. At the same time, this study also has a key exclusion criterion, such as "being a medical student."

Data Collection, Tools, and Procedure

The data collection consisted primarily of qualitative and quantitative data. Qualitative data was the predominant form throughout the research study. This consisted of the participants' responses from the ongoing surveys. As for quantitative data, this came through the usage of qualitative data. Based on the responses received from qualitative data, quantitative data was used to measure the overall agreement and consensus amongst participants' answers and determine the alignment with answering the research question. When comparing the process of data collection amongst other similar studies, this study was different. Studies done by Sharma et al. and Barry Rubin et al. showed the usage of only quantitative survey tools, such as the WBI and MBI, rather than the combination of quantitative and qualitative study approaches that were used for this study. After establishing how the data was collected, it was also essential to outline the tools that supported this process. For tools, the tools used to collect the data and information were relatively short. Participants answered each question in the Google survey, which then stored the answers in Google Sheets, which were then analyzed and interpreted in a Google document. Once the tools were established, the structured procedure was used to provide the best results from the participants. The majority of this study mirrored the process of a Delphi study. Using a Delphi study was the most logical way to answer the research question, as it was iterative, built consensus among the participants being surveyed, and was a safe way of conducting research by keeping all participants out of danger and being anonymous. Several studies helped provide examples of different procedures used to collect data on burnout. A study done by Thomas Reith, MD, used the Maslach Burnout Inventory (MBI) as the primary tool for measuring burnout. These studies provided the foundational stepping stones through the different ways to approach and address burnout amongst cardiologists.

Ethical Research Procedures

Four ethical research procedures were utilized for this research study. Honesty, confidentiality, security of people involved in scientific research, and social responsibility. First, all findings were accurately presented. No results were altered or changed. Second, participants remained anonymous. All participants were kept anonymous, and no additional information was given that could lead to the whereabouts of any of them. Third, the security of people involved in scientific research: all procedural steps were taken with the insight of minimizing damages and risks and maximizing benefits and results. All participants were put under no pressure or stress

throughout the ongoing rounds of the survey. Finally, social responsibility. Upon receiving answers from participants, every tool and resource was utilized to produce and provide this study's findings to others in this field and to those who are not. With all of these in perspective, two key studies provided examples highlighting the key ethical practices in research. The researcher, Jenner et al., demonstrated the process of informed consent, stating that "only 212" consented to participate from the "398 responders." And to further add another reason, Rubin highlighted the ethical importance of confidentiality. Stating that the reason many participants were willing to participate in their study and not others was because of the anonymity and confidentiality provided and affirmed by their study.

Limitations and Analysis Strategies

There were a few limitations associated with this study. One of the biggest limitations of this study was the overall religious backgrounds of the participants. All of the cardiologists who participated in the research study were Muslim doctors, which hindered the overall accurate sample representation. Additionally, the sample size in this study was not large enough to yield results representative of the entire population. The usage of only 10 cardiologists weakens the accuracy of the expressions of this study towards the overall population of cardiologists. Similarly, many research studies prior to this also identified the lack of population in cardiologists surveyed. Sharma et al. state, "Our study sample was limited to cardiologists captured in the ACC database and those cardiologists with an interest in this topic, and, as such, may not be representative of the experience of the field as a whole." Both this study and multiple other studies acknowledge these limitations, yet still proceeded with the study, as the results were beneficial and insightful to the cardiology community. On the contrary, the analysis and interpretation of the data posed minimal limitations through a two-step process. Combining both qualitative and quantitative methods, qualitative analysis was used for analyzing and identifying themes from the participants' responses, and after analysis and identification, quantitative analysis was used to measure the overall agreement and consensus among the participants. To further include, 2 distinct sources helped provide models for a thematic analysis of data. The first source by Alexandrou et al. provided a clear thematic framework by separating it into three parts: 1. the percentage of burnouts, 2. the factors of burnout, and 3. The solution to burnout.

Results

On the grounds of answering the research question, a Delphi study was deemed the most optimal method for getting an answer to this research question. The Delphi study consisted of three different rounds, with each round consisting of open-ended questions. Furthermore, the experts seemed justifiable, for this study consisted of many different subspecialties in the field of cardiology. Through having interventional cardiologists, general cardiologists, electrophysiologists, pediatric cardiologists, and cardiologists subspecializing in heart and vascular care, a clear and thorough consensus was established in this Delphi study.

In terms of how the results were generated from the Delphi study, each distinctive round shaped a critical component in answering the research question. For round 1, cardiology experts were not answering preset statements. They were responding to abstract and novel questions that could then form the interconnectedness of recurring themes to address in the second round. For round 2, the raw qualitative data were used to ask much deeper questions. This was possible through the usage of thematic analyses, which helped refine and clearly identify emerging themes among the experts' responses. Finally, round 3 was composed of confirmation and consensus affirmation. Round 3 focused on experts evaluating compiled sentences that discussed the extent of contribution of key topics that had emerged throughout the 2 previous rounds. Such thresholds were defined as strong agreement, moderate agreement, etc.

Table 1 (mentioned below) provided the three prominent categories for differentiating between the abundance of themes that were established. The three categories consisted of health care system factors, emotional factors, and individual factors. Similarly, a study done by Stanford physician Shanafelt et al. also organized physicians' burnout themes into extensive categories, such as organizational and individual fields, showcasing the trends of the Delphi study in perspective to much larger studies. As shown in Table 1, several ongoing themes categorized for burnout emerged and were vigorously restated in the continuations of the rounds. Themes that fell under the health care system in Table 1 were primarily administrative burden and high workload. As for the categories, emotional and individual, the accumulation of emotional strain over time was best identified for the emotion category, and for the individual category, coping resources that an individual could implement and use was the best approach from the individual category. In addition, almost all of the key topics under each category were progressively strengthened and agreed on from the consensus of the experts.

Table 1
Key Themes Related to Burnout Identified Across Delphi Rounds

Theme Category	Specific Theme	Round 1	Round 2	Round 3	Level of Agreement
Health Care System	Administrative burden	Identified frequently	Reinforced	Confirmed	Strong consensus
Health Care System	High workload and long hours	Identified frequently	Reinforced	Confirmed	Strong consensus
Emotional	Emotionally challenging patient outcomes	Mentioned	Expanded	Emphasized	Moderate consensus
Emotional	Accumulation of emotional strain over time	Emerging	Clarified	Confirmed	Strong consensus

Health Care System	Lack of institutional emotional support	Mentioned	Clarified	Emphasized	Strong consensus
Individual	Coping resources and persistence	Mentioned	Expanded	Contextualized	Moderate consensus

Summary of primary themes contributing to burnout among cardiologists through the three rounds of the Delphi study.

Several key primary stressors were identified as the most prevalent and frequently associated stressors with burnout. The cardiologists stated these stressors as being administrative burden and long workload and work hours being the biggest contributors to burnout, while the emotional toll of patient care and lack of health care were stressors identified as things that were now being seen as contributors to burnout (Table 3). Throughout the iteration of the study, burnout was continuously established by the cardiologists as a characterization primarily made up of administrative burdens, long workloads, and work hours (Table 1). Furthermore, those 2 stressors were profoundly identified and agreed upon by the consensus of the cardiologists. Similarly the American College of Cardiology conducted a study of 2313 cardiologists and fellows and concluded that cardiologists who were working more than 60 hours a week reported higher rates of burnout, thus strengthening one of the key contributors found in the Delphi study: long hours and workload. However, Alexandrou et al.'s narrative review of existing cardiology burnout literature disagrees. Through the synthesis of many existing studies in cardiology, Alexandrou et al. found that the major contributors to burnout stemmed from “computerization of practice, hostile and hectic work environments, and insufficient income,” rather than administrative burdens and excessive workload being the primary contributors, hence providing another viewpoint towards this conversation.

Table 3
The Primary Stressors That Contribute to Burnout in Cardiology

Burnout Contributor	Expert Response (Across Rounds)
Administrative burden	Major contributor
Workload and hours	Major contributor
Emotional toll of patient care	Significant to major contributor
Lack of healthcare support	Increasing factor

Primary contributors of burnout according to cardiologists.

The key purpose of this study was to compile and identify a consensus among the cardiologists, which helps in answering the research question. While many responses brought new results to the topic, the primary purpose of the study proved crucial in answering the research question. For round 1 (Table 2), several different questions asking about the emotional toll led to mixed responses by the cardiologists. Particularly speaking, in round 1, the overall agreement seemed to be that emotional toll was framed as secondary or event-based, with even some disagreements amongst each other. However, in round 2, after the questions were modified and refined to target the specific aspects of emotional toll mentioned by the cardiologists, emotional toll was clearly recognized as cumulative emotional strain, leading to momentous strengthening in opinions. Conclusively, in round 3, with the last refinement being done, emotional toll was identified by each cardiologist as a significant contributor. Not only did the progression of the rounds help provide a deeper and clearer thematic answer towards the research question, but it also helped provide further novel aspects of emotional toll that the majority of experts deemed as deleterious. In comparison to the Delphi study done in this research paper, several researchers from different academic journals proved similar conclusions. Findings from these researchers suggest that the emotional aspects of burnout progressively worsen over time rather than occurring as a single event (Shanafelt and Noseworthy; West et al.). Therefore, proving the statistical significance of the results that stemmed from this area.

Despite the positive consensus throughout the progression of the rounds, there still laid a clear divergence in perspectives on the emotional toll. Even after the completion of round 3, there was still no clear agreement amongst the experts on whether or not emotional toll was a contributor of its own and on how the significance of emotional toll ranked in comparison to other stressors of burnout.

Table 2
Progression of Expert Perspectives on Emotional Toll and Burnout Across Delphi Rounds

Topic	Round 1 Characterization	Round 2 Clarification	Round 3 Stance
Role of emotional toll	Often secondary to workload	Accumulation over time leading to become a massive contributor	Significant contributor
Nature of emotional strain	Event-based	Repeated exposure over time	Accumulative and compounding
Relationship to administration	Separate stressors	Interacting stressors	Interdependent contributors
Responsibility for burnout	Mixed (individual/health care)	Increasingly health care	Predominantly health care
Addressability	Unexpressed	Conditional	Preventable with support

Illustrates merging of expert opinions regarding the contribution of emotional toll to burnout across the Delphi rounds.

Analysis

The main goal of the research paper was to best answer the research question: to what extent does the emotional toll of patient care contribute to burnout among physicians in the field of cardiology? My Delphi study measured overall consensus and clarification regarding the notion of emotional toll as a contributor to burnout. Based on the Delphi study, several statistically significant findings emerged and thus provided new conventional wisdom in regard to the field of cardiology.

Contextualizing the aspect of emotional toll, several statistically significant results provided insight into current views pertaining to emotional toll. The results of the Delphi study helped explain and synthesize why the emotional toll matters based on the results found. One key finding that resulted in the form of a table was the progression of expert perspectives on emotional toll throughout the Delphi rounds. Numerically called table 2, it shows that throughout the duration and rapid progression of the rounds, the emotional toll becomes refined as it shifts from a secondary contributor to a significant contributor by the end of the round, according to the cardiologists. Furthermore, Table 2 helps provide not only the characterization of emotional toll but also its clarification. This helps simplify and explain the technical terms better. Also, on a larger scale, Stanford and University of Colorado medical school professors Shanafelt and Noseworthy contend that burnout is driven by long-term emotional exhaustion. This helps support the findings seen in the Delphis and thus strengthens the validity of the study. The medical school professionals also state that addressing and potentially solving burnout requires acknowledging ongoing emotional strain, thus providing stronger contentions in relation to the emotional toll.

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Illustrates merging of expert opinions regarding the contribution of emotional toll to burnout across the Delphi rounds.

Although the research paper and expectations were to analyze and interpret results in accordance with emotional toll, the discovery was more in the direction of serendipity. Initial assumptions heavily favored emotional toll as being the primary result; however, another crucial, yet key, aspect emerged from this research. The ultimate statistically significant findings were in relation to administrative burden being the primary driver of burnout. Such findings were clearly seen in Table 3. Perhaps the most important table of them all, it simplified the different contributors and their significance towards the emotional toll based on the multiple rounds aimed at achieving consensus. This contributor was the clear winner. Alongside workload and hours throughout all three rounds, administrative burden was identified by at least 5 of the 8 cardiologists. In comparison to other studies pertaining to this topic, these results are not surprising. According to the American College of Cardiology, burnout among cardiologists was closely related to extended work hours from administrative burden. Hence, supporting the findings. However, based on a meta-analysis done by physicians West et al., burnout was characterized by several interacting contributors, such as emotional toll, rather than primarily being an administrative burden. This analysis hence provides ambiguity and confusion in terms of coming closer towards identifying the common contributor, as there are many conflicting views, and thus calls for future refinements and additional iterative consensus amongst each other.

Table 3
The Primary Stressors That Contribute to Burnout in Cardiology

Burnout Contributor	Expert Response (Across Rounds)
Administrative burden	Major contributor
Workload and hours	Major contributor
Emotional toll of patient care	Significant to major contributor
Lack of healthcare support	Increasing factor

Primary contributors of burnout according to cardiologists.

In hindsight, several things were identified that resulted in gaining a unique and advantageous conveyance in respect to others. The notion that this paper utilized a Delphi study, while many others did not, provides a serious advantage. When collecting data for analysis, it is not only crucial but critical to accumulate convergence and consensus amongst all participants in helping answer your study's question. For example, while West et al. quantified burnout occurrence and demonstrated many realistic mitigation ideas, their survey-based approach did not explore or provide answers in regard to what physicians themselves had to say, meaning physicians never shared their personal opinions; rather, they merely answered questions. In contrast, my Delphi study demonstrated personal opinion, consensus, and clarification regarding the many different progressions for burnout and, more importantly, its relation to the emotional aspect of it.

When evaluating the benefits and determinants of conducting a research study, it's imperative to understand the extent of novelty and importance to the community. For my research, after delving into a deeper analysis of basic problems associated with burnout among staff in cardiology, I found it important and necessary to conduct my research. The results of this research proved to have impacted cardiologists, the American healthcare system, and future researchers. This research conceptually changed how current researchers view and execute studies. It also helped shift burnout in a direction that, when evaluated, will provide much more crucial findings that will help potentially solve or, at a minimum, decrease the likelihood of cardiologists getting frequently burnt out.

Throughout the statistically significant results, several consensuses emerged, yet at the same time, there were several disagreements and divergences amongst responses. Some of the biggest divergences that were identified in the results of this study were why the emotional toll ranking remained unclear, why experts may have struggled to separate emotional toll from workload, and how this reflects broader challenges in burnout research. After doing qualitative data analysis and pattern identification, multiple grounds became established, and the reasoning for why several contributors had become the result was visible. To begin, if I had used numerical identification for contributors and limited the authorization of open-ended questions, such results would have been more direct. To also add in perspective, West et al. came to the definition of burnout as multidimensional, hence strengthening the divergence and variety of stressors identified. Furthermore, West et al. showed burnout as an involvement in interacting components, which would help explain the vast amount of stressors that came in my results.

Conclusion

Summary

Returning to the question posed at the beginning of this study, it is now possible to state that although emotional toll may be a factor in creating burnout, it is critical to state and further research the bigger causes that were stated by experts as the major contributors towards burnout: administrative burden, and greater workload and hours. The serendipity of the major contributors

identified through the consensus of the experts are clearly supported by current findings in today's research. Tebra supports the notion that excessive workload causes a significant burden by stating, “Documentation and 'EHR inbox overload' require extensive evening and weekend work.” Furthermore, Murj supports the other finding of administrative burden being a significant contributor to burnout. He states, “Significant administrative workload is an all-too-common cause of burnout,” thus showing that current research amongst experts is already in agreement with these widely identified contributing factors.

Limitations

The findings in this report are subject to at least four salient limitations. First, the limited sample size. Originally 13, then reduced to 8, the numerical population represented is far from the actual population required to produce statistically significant contributions to society. In respect to my research, the Mehta et al. study used data from over 10,000 cardiologists to analyze burnout, hence showcasing the widely different population sample size. Second, a limitation was seen with the usage of methodical open-ended Delphi-style questions. At the start, 13 participants consented and participated in round 1; however, by rounds 2 and 3, only 8 stayed in the study. Because of the usage of long, open-ended response questions, participants didn't have the time to keep participating in the study and consequently dropped out of the study, grievously reducing the sample size of this study. Third, the selection criteria applied for this study are clearly flawed. All of the participants who participated in this study were not only male but also from a single ethnicity. Having only male participants unequally represents the other gender's perspective. Furthermore, having only one ethnicity results in a cultural cohesion that could seriously cause overt dominant opinions and perspectives when analyzing the findings stated by them. Finally, the last and final major limitation was the relationship that the participants had with the experimenter. All the participants knew the experimenter and were friends with him. This limitation means that study findings need to be interpreted cautiously so that researchers can clearly see major obscurities and deficiencies when analyzing and basing off of this paper.

Implications and Potential Impact

Several audiences benefit from these statistically significant findings. Such audiences include current practicing cardiologists and researchers studying burnout in the medical space. Although the results produced provide serious insight into burnout and its top contributors, the consensus achieved minimally provided a mitigation plan for how to act on current problems, hence creating a futuristic implication for future research. In future studies, therefore, it seems that researchers and experimenters need to further clarify and state such mitigation plans to be enforced for providing solutions to such catastrophic contributors for burnout. The results produced expand the current research in burnout amongst cardiologists by reinforcing and restating the most common contributors. Furthermore, from a long-term perspective, this

research will prove beneficial in supporting current theories by providing further strengthening evidence.

Future Research Avenues

From a protracted lens, future studies can expand on these findings by targeting the specific contributors identified in this paper. By establishing the parameters before the study, it will be clear to all participants what must be further evaluated to provide deeper insights into the medical field in relation to burnout. Ideally, these findings should be replicated in a study where there is an abundance of various types of cardiologists, and they are easily accessible. Additionally, throughout the duration of this study, several key gaps were identified. However, the preeminent one being the lack of explanation behind each identified contributor. If a deeper and prolonged duration had occurred in the experiment, more major refinements could have been made, thus allowing for a clearer and more specific understanding of such causes of burnout. Finally, with respect to suggested methodologies, the idea of a specific one-time survey could be plausible for future research; however, it could also have limitations by not being able to form iterative consensus, like what was presented in this study.

Connecting Back to the Thesis

Originally, the goal was to study the aspect of emotional toll from patient care in regard to burnout. However, throughout the progression of the rounds, it was clear that there were other, more important contributors that need to be further researched in the future. This study helped researchers and experimenters gain a different way of collecting data that was still beneficial, if not more beneficial, in helping identify the biggest contributors to burnout.

Call To Action

To conclude everything, the experiment that led to the creation of this paper proved successful and beneficial for the cardiology community. By utilizing a novel research method with multiple different types of cardiologists, it's safe to say that the results that came from this experiment are not only statistically significant but also provide and support critical information in the current understanding of burnout. Upon viewing this paper, I look forward to others gaining valuable insights and finding the courage to go and conduct a study of their own that can be just as, if not more, contributive to society.

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From Food to Molecules: Molecular Mechanisms Linking Diet to Brain Function
By Zihan Zou

Abstract

Various nutrients obtained from food provide molecules that link diet to brain function. This research paper explores how different macronutrients and micronutrients link food to brain function. Carbohydrates obtained from diet help in brain function by providing glucose, the primary source of energy for the brain. Metabolism of glucose through glycolysis and channeling of the subsequent metabolites to the TCA cycle provides reducing equivalents used in the electron transport chain to generate ATP, the cellular form of energy. Fermentation of dietary carbohydrates by the gastrointestinal microbiota is also associated with production of short-chain fatty acids that have neuroprotective effects. Dietary proteins, on their part, provide essential amino acids used to synthesize such neurotransmitters as serotonin and histamine. Proteins also participate in the maintenance of neurons to ensure optimal cognitive function. Fats are core components of membrane neural cells and some such as polyunsaturated fatty acids have roles in neuronal homeostasis and synaptogenesis. Further, fats are sources of ketone bodies, alternative sources of energy for the brain when glucose availability is low, such as when glucose transporters are impaired. Vitamins and minerals are important antioxidants that prevent damage to the cells of the brain, with minerals also having a role in oxygen transport and energy generation by being part of proteins that facilitate such roles. Such a role of nutrients in the optimal functioning of the brain explains why some of the interventions to address cognitive decline in diseases such as Alzheimer's disease target the diet.

Keywords diet, Brain function, gut-brain axis, carbohydrates, proteins, fats, vitamins, minerals, gut microbiota.

From Food to Molecules: Molecular Mechanisms Linking Diet to Brain Function

Diet plays a multifaceted role in brain function, with various macronutrients and micronutrients derived from diet contributing to the structure and function of the brain. Human brains, for instance, have a high energy demand, estimated to account for 25% of glucose utilization and 20% of oxygen consumption despite comprising only about 2% of body weight (Bordone et al., 2019). Foods that provide the needed molecules for energy metabolism are thus important for brain function. Similarly, apart from providing structural components to the neural cells, amino acids derived from dietary proteins are precursors for the synthesis of neurotransmitters, the chemicals that facilitate the transmission of nerve impulses (Gao et al., 2020). Fats also have a role in brain function, contributing up to 50% of the brain's dry weight and forming critical components of the cell membranes, including those of the neurons (Chianese et al., 2018). Vitamins, one of the micronutrients obtained from food, also have important roles in brain function such as providing antioxidant activity that prevents the oxidative degradation of neurons (Rai et al., 2021). Further, minerals such as magnesium regulate the release of

neurotransmitters and modulate inflammation of the neurons, and thus their deficiency can lead to cognitive decline (Patel et al., 2024). These examples illustrate how various nutrients obtained from food facilitate brain function, and the subsequent sections discuss how molecules obtained from these nutrients link diet to brain function.

Dietary Carbohydrates and Brain Function

Dietary Carbohydrates as a Source of Metabolites for Energy Metabolism

For energy metabolism, the primary metabolite obtained from carbohydrates is glucose. Glucose is obtained from carbohydrates through their breakdown in the mouth by salivary amylase and by other enzymes in the remainder of the gastrointestinal tract (Holesh et al., 2026). Breakdown of carbohydrates into such monosaccharides as glucose and fructose enables their absorption into the bloodstream through such specialized transporters as sodium-glucose linked transporter 1 (SGLT1) and glucose transporters (GLUTs; Nelson et al., 2021). At the blood-brain barrier, various isoforms of GLUTs (1-14) and SGLTs (1-12) exist, and these enable glucose in the blood to cross into the brain (Patching, 2017). Entry of glucose into the brain enables its use in energy metabolism within the neurons. Such a role of glucose in providing energy for the brain explains why the brain contributes up to 25% of glucose utilization in the body (Bordone et al., 2019) and why eating foods that lead to a sharp rise and a subsequently fast fall in blood glucose levels could lead to decline in cognitive function due to inability to sustain glucose levels the brain requires for its optimal functioning (Arshad et al., 2025).

Once inside the cells, glucose is principally metabolized through glycolysis. Glycolysis is a stepwise, enzyme-catalyzed breakdown of glucose (a six carbon sugar) into two molecules of pyruvate (three -carbon molecule), two ATP molecules, and two NADH molecules as illustrated in Figure 1 (Nelson et al., 2021). This pathway occurs in the cytosol (Nelson et al., 2021), and while it does not provide most of the energy (ATP) requirements of the cell, it provides metabolites channeled to other energy-generating pathways.

One of the fates of the pyruvate molecules from glycolysis is their channeling to the tricarboxylic acid (TCA) cycle. Since glycolysis occurs in the cytosol while TCA occurs in the mitochondrial matrix (Nelson et al., 2021), the pyruvate generated in the cytosol is transported across the outer mitochondrial membrane by voltage-dependent anion channel and across the inner mitochondrial membrane by the mitochondrial pyruvate carrier (Zangari et al., 2020). On entry into the mitochondrial matrix, each pyruvate molecule is oxidized to a molecule of acetyl CoA, through the activity of pyruvate dehydrogenase, in a step that releases carbon dioxide, synthesizes a molecule of NADH and requires coenzyme A as a cofactor (Zangari et al., 2020). The produced acetyl CoA enters the TCA cycle through the activity of citrate synthase, which combines the acetyl CoA with oxaloacetate to form citrate (Nelson et al., 2021). After each cycle, each acetyl CoA molecule that entered the cycle will have generated one GTP (ATP) molecule, three NADH molecules and one $FADH_2$ molecule and will have released two carbon dioxide molecules as shown in Figure 1 (Nelson et al., 2021).

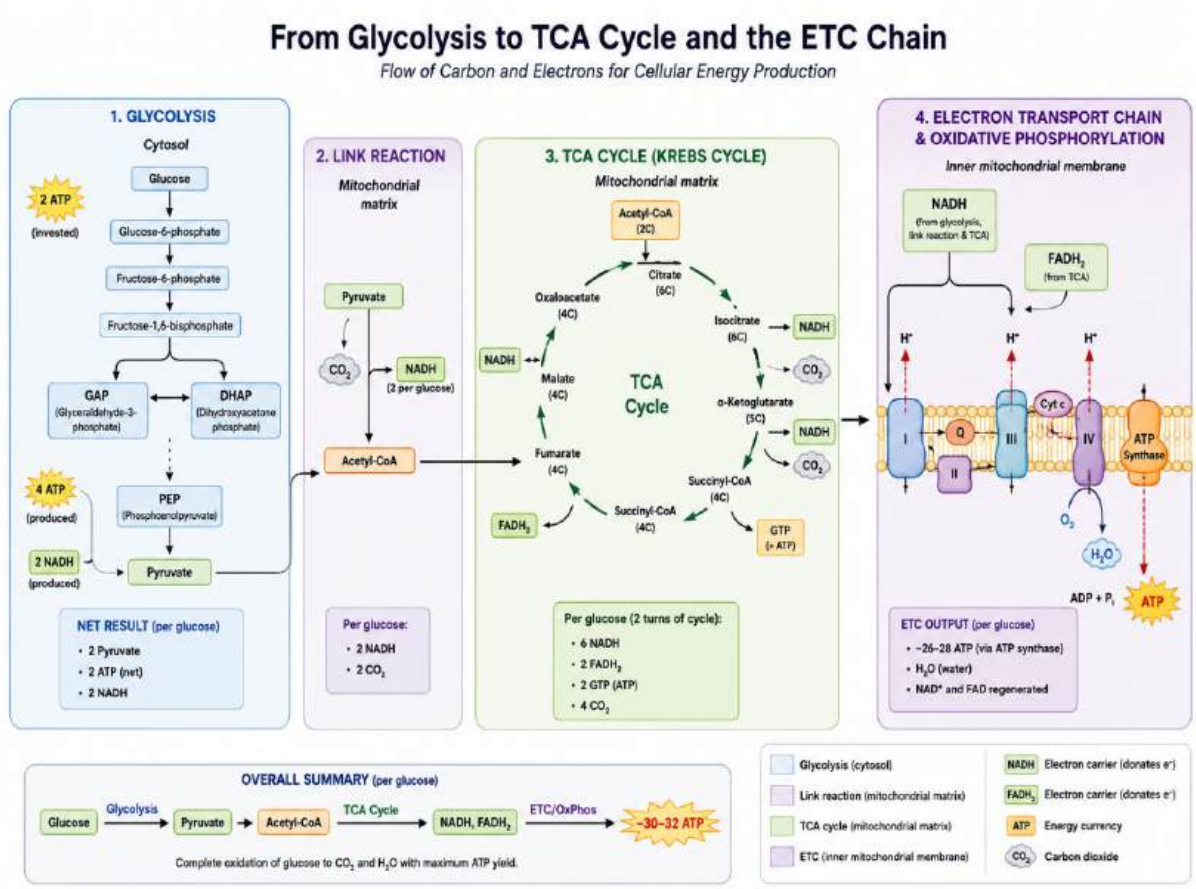


Figure 1. How carbohydrates from diet generate energy needed by neurons. Glucose from the breakdown of dietary carbohydrates provides ATP directly through its breakdown in glycolysis and channeling of the metabolites to TCA cycle. These pathways also generate reducing equivalents (NADH and $FADH_2$) that are channeled to the electron transport chain (ETC), which generates the bulk of ATP molecules through the ATP synthase. Image developed using ChatGpt v5.5.

While both glycolysis and the TCA cycle provide ATP molecules directly, most of the ATP is generated in the electron transport chain (ETC). The ETC occurs in the inner membrane of the mitochondria and involves the oxidation of the reducing equivalents (NADH and $FADH_2$) in a chain of reactions that create a proton gradient across the inner mitochondrial membrane as electrons pass through a chain of protein complexes that are integrated into the inner membrane (Nelson et al., 2021). At the end of this chain, the enzyme ATP synthase uses this gradient to synthesize ATP as illustrated in Figure 1 (Nelson et al., 2021). Each molecule of NADH is associated with synthesis of 2.5 molecules of ATP whereas each molecule of $FADH_2$ is associated with synthesis of 1.5 molecules of ATP (Hinkle, 2005). As such, if all the reducing equivalents associated with the metabolism of one molecule of glucose are channeled to the ETC, the ETC would generate up to 28 molecules of ATP as illustrated in Figure 1 below. This results in a maximum output of 32 ATP molecules from one glucose molecule after adding the other ATP molecules generated in glycolysis and TCA cycle.

Dietary Carbohydrates Affect Availability of Tryptophan for Synthesis of Serotonin

Apart from acting as energy sources for the brain, dietary carbohydrates also have a role in regulating the synthesis of serotonin (5-hydroxytryptamine, 5-HT). Serotonin is a neurotransmitter that regulates such aspects such as appetite, mood, anxiety, sleep and sexual behavior (Nonogaki, 2022; Walther et al., 2003). Within the brain, 5-HT is synthesized in the brainstem through the activity of tryptophan hydroxylase 2 (TPH2), an isoform of the TPH1 enzyme that is responsible for synthesis of 5-HT in the gut (Walther et al., 2003).

The role of dietary carbohydrates on the synthesis of serotonin has been demonstrated in various studies. For instance, by stimulating the release of insulin, dietary carbohydrates signal for the decrease in the plasma concentration of branched amino acids but not of tryptophan, the precursor for serotonin synthesis (Wurtman et al., 2003). Such effects raise the plasma tryptophan ratio, which leads to a concentration gradient that favors its entry into the brain to facilitate its use in the synthesis of serotonin through the activity of TPH2 (Wurtman et al., 2003). Further, as Gao et al. (2019) showed, increased availability of carbohydrates in the intestines lowers the metabolism of aromatic amino acids such as tryptophan by gut-resident microbiota. Such lower metabolism of these amino acids increase their availability in the blood and thus facilitate entry of tryptophan into the brain for the synthesis of serotonin (Gao et al., 2019). As these examples reveal, dietary carbohydrates thus play a role in the availability of tryptophan in the brain, and thus in the synthesis of serotonin, a neurotransmitter responsible for various activities of the brain.

Carbohydrates Affect the Gut-Brain Axis

Apart from their effects on metabolism of amino acids by gut microbiota, dietary carbohydrates also affect the gut-brain axis when they are fermented by the gut flora. Fermentation of these dietary carbohydrates results in generation of short-chain fatty acids (SCFAs), which have been shown to have neuroprotective roles (Schneider et al., 2024). Most of these SCFAs are obtained when microbiota ferment dietary fibers that are indigestible by the host, and these fibers are obtained from fruits and vegetables that contain such carbohydrates as fructooligosaccharides and galactooligosaccharides (Schneider et al., 2024). The role of these carbohydrates in the synthesis of SCFAs and the subsequent neuroprotective effects of these SCFAs thus provide an additional link between carbohydrates and brain function through the gut-brain axis.

The role of carbohydrates in the gut-brain axis is also supported by observations of mental effects arising from different types of carbohydrates. For instance, diet comprised of complex carbohydrates derived from fruits, vegetables and whole grains, which is associated with a balanced microbiome in the gastrointestinal tract (Schneider et al., 2024), is associated with improved mental health and lower risk of inflammation (Arshad et al., 2025). Conversely, simple-sugar diets and highly refined carbohydrates are linked to an imbalanced microbiota, that contribute to increased inflammation, higher intestinal permeability, and development of dangerous bacteria (Arshad et al., 2025). Such shifts within the gastrointestinal tract have, in

turn, been linked to such mental issues as anxiety, impaired cognition, and mood disorders (Arshad et al., 2025). These examples thus show that carbohydrates also have an indirect effect on the brain through their effect on the microbiota that then influences the gut-brain axis.

Dietary Proteins and Brain Function

Dietary proteins play critical roles in the brain because they offer amino acids that are used in the synthesis of proteins needed for the structure and function of the neurons. The role of diet in availing such amino acids arises because some of the amino acids, known as essential amino acids, cannot be synthesized within the body and thus must be obtained from the diet (Nelson et al., 2021). These essential amino acids include tryptophan, valine, threonine, isoleucine, histidine, leucine, phenylalanine, methionine and lysine (Schneider et al., 2024). Some of these amino acids are important precursors for the synthesis of neurotransmitters, whereas others are important building blocks for proteins that form structural components of the cells of the nervous system.

Proteins as Sources of Amino Acids Required for Neurotransmitter Synthesis

A central role of dietary proteins is the provision of amino acids needed for the synthesis of different neurotransmitters, the chemical substances that enable the transmission of nerve impulses across the synapses. Figure 2 for instance illustrates various neurotransmitters that are derived from amino acids. Gamma-aminobutyrate (GABA) does not rely on dietary sources because it is synthesized from glutamate, a non-essential amino acid (Nelson et al., 2021). Similarly, dopamine and epinephrine (adrenaline), which are derived from the sequential hydroxylation and decarboxylation of tyrosine, do not rely directly on dietary sources because tyrosine is not an essential amino acid (Nelson et al., 2021). However, these rely indirectly on diet because tyrosine is synthesized from phenylalanine, an essential amino acid (Nelson et al., 2021). As shown in Figure 3, the synthesis of tyrosine from phenylalanine is catalyzed by phenylalanine hydroxylase (Nelson et al., 2021), and thus individuals in whom this enzyme is deficient, i.e., those who suffer from phenylketonuria (PKU), need diets rich in tyrosine for them to effectively synthesize dopamine and epinephrine. As such, whereas these neurotransmitters are not directly dependent on dietary proteins, they have an indirect link to such proteins. The other neurotransmitters illustrated in Figure 2 depend entirely on dietary proteins because they are synthesized from essential amino acids. For instance, histamine is synthesized from the decarboxylation of histidine whereas serotonin is synthesized from the sequential hydroxylation and decarboxylation of tryptophan (Nelson et al., 2021).

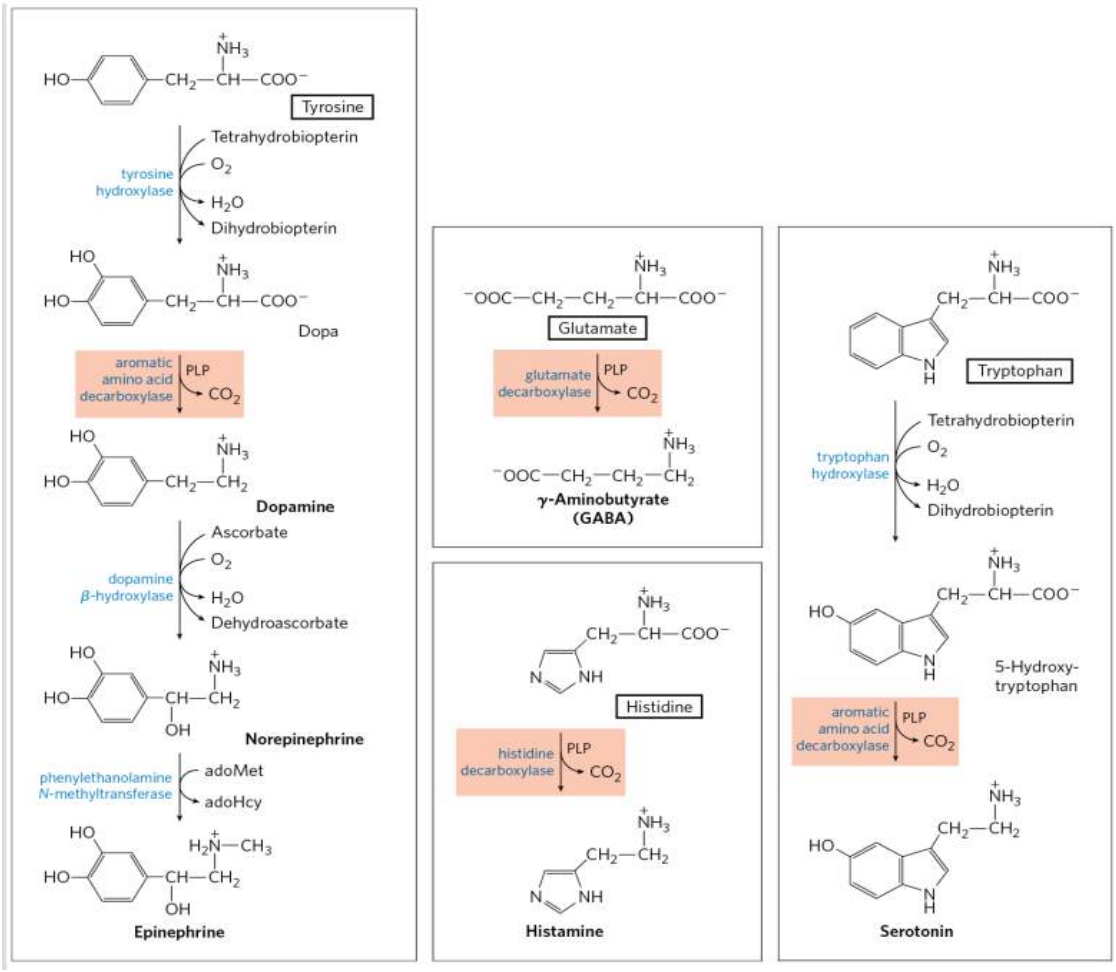


Figure 2. Neurotransmitters synthesized using amino acids as precursors. Source: Nelson et al. (2021, p. 2878).

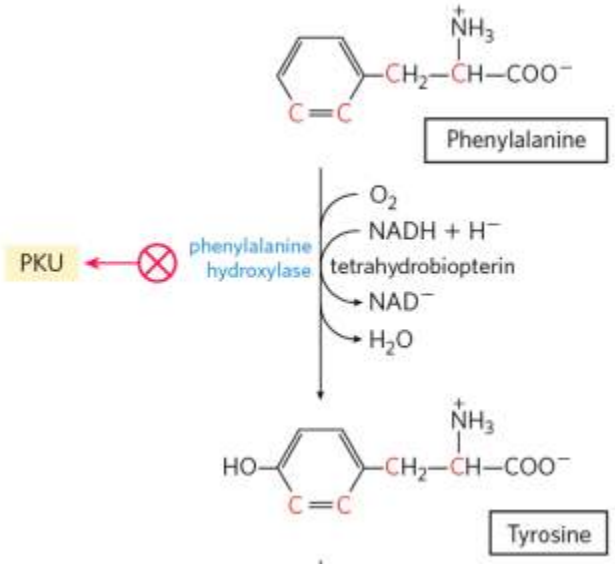


Figure 3. Synthesis of tyrosine from phenylalanine within the human body. Source: Nelson et al. (2021, p. 2327).

The link of dietary proteins to brain function through their role in synthesis of neurotransmitters can be exemplified by the role of tryptophan. As an essential amino acid, tryptophan is released in the small intestines following digestion, then absorbed into the blood through a sodium-dependent transporter (Gao et al., 2020; Nelson et al., 2021). Within the blood, 80-90% of the tryptophan circulates bound to albumin with the unbound form contributing to protein synthesis, synthesis of serotonin (which further acts as a precursor for synthesis of the pineal hormone melatonin) or is degraded through the kynurenine pathway (Gao et al., 2020). Synthesis of serotonin occurs in both the gastrointestinal tract and the brain under the activity of different isoforms of tryptophan hydroxylase, TPH1 and TPH2 (Walther et al., 2003). In terms of proportions about 90% of the serotonin is synthesized in the gastrointestinal tract especially in the enterochromaffin cells located in the hindgut, with only 10% being synthesized in the brain (Gao et al., 2020). These two pools of serotonin are usually kept separate by the blood-brain barrier that prevents the crossing of serotonin (Gao et al., 2020), and thus the synthesis within the brain is favored when the plasma tryptophan ratio increases (Wurtman et al., 2003). Such an increase in plasma tryptophan ratio is influenced by diet, including both carbohydrates and proteins that affect the microbiota metabolism in the gastrointestinal tract (Schneider et al., 2024; Wurtman et al., 2003). As the example of tryptophan thus reveals, dietary proteins are critical determinants of availability of neurotransmitters such as serotonin that then enable the brain to perform various functions through their role in facilitating transmission of nerve impulses.

Proteins for Maintenance of the Neurons

As structural components of various cells including neurons (Nelson et al., 2021), proteins also play a role in maintenance of neurons and thus their activity. For instance, a longitudinal study by Cui et al. (2024) illustrates how dietary proteins may have implications on the structure markers in the brain. Based on their survey, the authors found that individuals who reported a historically higher consumption of animal protein had lower reduction of total hippocampus volume and left and right hippocampus volumes. This lower reduction in hippocampal volume was attributed to lower atrophy of cells in individuals with a higher consumption of animal protein (Cui et al., 2024). Such findings illustrate the role of proteins in neuronal maintenance, helping retain healthy neurons that can delay cognitive decline.

Dietary proteins could also aid in maintenance of healthy neurons through their effect on amyloid-β (Aβ) deposition. Aβ is a self-aggregating protein obtained from the cleavage of amyloid precursor protein (APP) and whose deposition in neurons has been associated with Alzheimer's disease (AD; Sehar et al., 2022). In healthy individuals, Aβ is associated with signal transduction processes, promoting neurite outgrowth, and facilitating neurogenesis (Sehar et al., 2022). However, when Aβ levels in the brain are dysregulated, insoluble plaques that are associated with AD arise (Sehar et al., 2022). Dietary proteins have been shown to reduce the formation of Aβ plaques in some studies. For instance, in a cross-sectional study that evaluated how self-reported protein intakes was related with Aβ deposition, Fernando et al. (2018) showed that the odds of having a higher Aβ burden were higher for those whose self-reported protein

intake was in the lower and middle tertile compared to those in the highest tertile. Such results illustrate that moderate to high protein diets could have beneficial effects in delaying the cognitive decline associated with Aβ deposition in those suffering from AD.

Dietary Fats and Brain Function

Fats form about 50% of the dry weight of the brain (Chianese et al., 2018), and they have multiple roles that affect brain function. For instance, in the event of glucose deficiency, such as during starvation, ketone bodies, which are derived from the oxidation of fatty acids, become the main source of energy (Romano et al., 2017). As Figure 4 shows, under such conditions as fasting, prolonged starvation, and diabetes, non-esterified long-chain fatty acids can cross the blood-brain barrier and enter into the astrocytes where they can be converted into fatty acyl-CoA (FA-CoA) that are then channeled to β-oxidation to form ketone bodies (KB; Romano et al., 2017). These ketone bodies, which include acetone, beta-hydroxybutyrate, and acetoacetate are then exported into the neurons where they can be converted into acetyl-CoA as shown in Figure 5 (Morris et al., 2020). The conversion of the KB into acetyl-CoA thus allows their entry into the TCA cycle as illustrated in Figure 4 (Romano et al., 2017), which can then enable them to be used for energy generation as discussed earlier for glucose (refer to Figure 1).

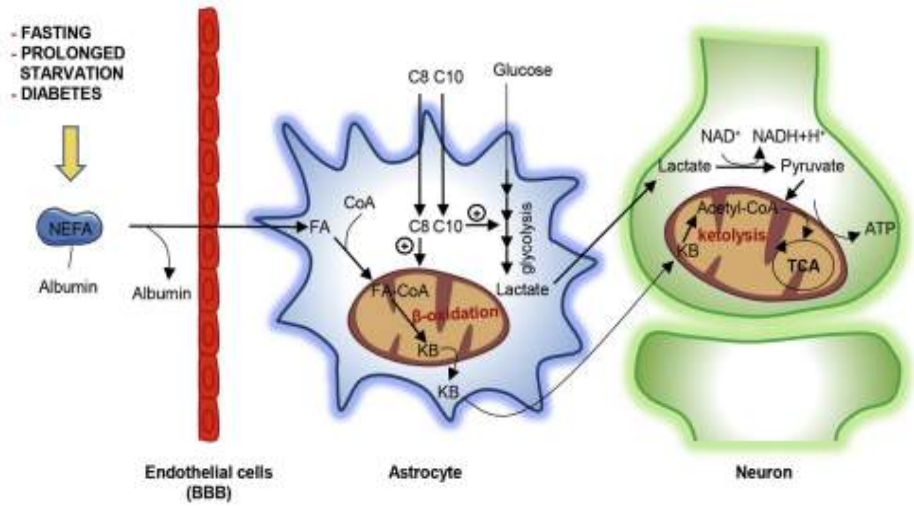


Figure 4. Fatty acids and energy metabolism in the brain. Source. Romano et al. (2017, p. 41).

Dietary fats play a role in these metabolic processes and thus enable different brain functions. Studies on ketogenic diets (diets having high fat and low carbohydrate content), have, for instance, been associated with improved cognition due to the increase in levels of GABA, a neurotransmitter (Romano et al., 2017). This increase in GABA is claimed to arise from the activity of glutamate decarboxylase, which is involved in the conversion of glutamate to GABA (Romano et al., 2017). The role of ketogenic diets in slowing the progression of cognitive decline in AD is also linked to such a KB pathway that offers alternative energy sources for the brain and thus reduces the effect of low availability of glucose due to the impairment of GLUT1 (Romano

et al., 2017). Further, the ketogenic diets are associated with lower levels of Aβ in animal models of AD (Romano et al., 2017). Even where the diet is not ketogenic, medium-chain fatty acids (those with 6-12 carbon atoms) that are found in many diets can be channeled into ketogenesis, leading to the benefits observed for use of fats as an alternative to glucose for providing energy to the brain (Romano et al., 2017).

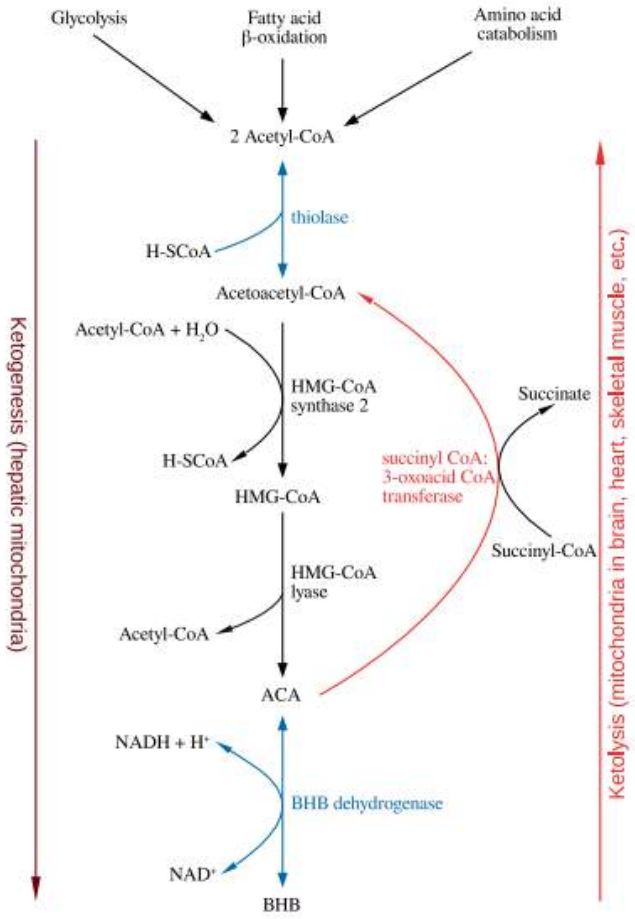


Figure 5. Pathways for generation of ketone bodies and for the generation of acetyl-CoA from ketone bodies. ACA, acetoacetate; BHB, β-hydroxybutyrate; HMG-CoA, β-hydroxy-β-methylglutaryl-CoA. Source: Morris et al. (2020, p. 2).

Polyunsaturated fatty acids (PUFAs) that can be derived from diet are also important in brain function. Among these PUFAs, Omega 3 ones such as docoesahexanoic acid (DHA) and omega 6 ones such as arachidonic acid (AA) are used to generate different endocannabinoids that are associated with brain function (Chianese et al., 2018). These endocannabinoids are linked to neuronal plasticity and synaptic activity that lowers the cognitive decline experienced during aging (Chianese et al., 2018). These PUFAs, especially AA and DHA, are incorporated into membranes of various neural cells, with AA having an even distribution in all types of neural cells whereas DHA is mainly distributed in neurons, including in the synaptic membranes (Romano et al., 2017). DHA has specifically been linked to various processes that sustain

neuronal homeostasis, including synaptogenesis, neuronal differentiation, neurite outgrowth, and membrane fluidity maintenance (Romano et al., 2017).

Dietary fats also contribute to the gut-brain axis through the influence of SCFAs on the metabolism of the microbiota. Within the gastrointestinal tract, the diet-derived SCFAs can regulate lipid metabolism with associated benefits of decreased plasma levels of free fatty acids (Schneider et al., 2024). Among these SCFAs, butyrate has been of interest since its production is based on the composition of the microbiota, which is in turn influenced by diet (Romano et al., 2017). Butyrate has critical roles in the body, because of its role as an inhibitor of histone deacetylase and thus is explored as a possible therapeutic tool for alleviating cognitive decline associated with impaired histone acetylation (Romano et al., 2017). SCFAs also have other neuroprotective roles (Schneider et al., 2024), illustrating how dietary fats could influence brain function through their modification of the brain-gut axis. As such, dietary fats have brain roles that include maintenance of nerve cell membranes, enabling synaptogenesis and thus nerve impulse transmission, offering alternative sources of energy to glucose, and reducing the damage of brain cells through inflammation.

Dietary Vitamins and Minerals and Brain Function

Vitamins are one of the essential micronutrients that humans have to get from diet since these cannot be synthesized in the body. A core function of vitamins is offering antioxidant activity and being cofactors for enzymes (Rai et al., 2021). Water-soluble vitamins such as thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), biotin (vitamin B7, vitamin H), folate (vitamin B9), and ascorbate (vitamin C) are critical antioxidants and cofactors used in different metabolic processes (Rai et al., 2021). Fat-soluble vitamins such as vitamin E are also strong antioxidants whereas others such as A and D control the expression of various genes (Rai et al., 2021). In terms of brain functions, vitamins, in their role as antioxidants, help in alleviating oxidative stress, which has been associated with the degeneration of neurons noted in such diseases as Parkinson's disease (Rai et al., 2021). Vitamin E intake has for instance been associated with a reduced risk of developing PD, with this effect linked to Vitamin E's antioxidant activity (Rai et al., 2021). Further, studies with various types of Vitamin B (Vitamin B9, Vitamin B12, and Vitamin B6) have shown that administration of these vitamins alleviates the symptoms of PD (Rai et al., 2021). This effect of Vitamin B is linked to its role in lowering the levels of homocysteine, a sulfur-containing amino-acid whose presence in high levels is associated with deterioration of dopaminergic neurons in the mid- and fore-brain (Rai et al., 2021). These examples show that vitamins have a protective effect on the brain cells, and thus link dietary consumption of vitamins to better brain function, especially the reduction of oxidative damage to brain cells that can lead to cognitive damage.

Dietary minerals are also essential nutrients since they cannot be manufactured within the body. Similar to vitamins, minerals contribute to various metabolic processes that are vital for brain functioning. For instance, iron is a core component of critical proteins such as hemoglobin and cytochromes (Tardy et al., 2020). As a component of these proteins, iron thus facilitates the

transport of oxygen needed by the brain (as part of hemoglobin) and enables the activities of cytochrome complexes that generate energy in the ETC (refer to Figure 1, ETC component; Tardy et al., 2020). Iron is thus an essential micronutrient and its availability in adequate amounts through diet enables availability of oxygen needed by the brain for respiration.

Apart from iron, other minerals also have implications for brain function through their roles in various processes. For instance, magnesium is a critical co-factor for enzymes that process glucose through the glycolytic and TCA cycles (Tardy et al., 2020). Further, ATP transport from the mitochondria to the cytosol is enabled by the formation of ATP-Mg complexes, which facilitate availability of this cellular fuel to other parts of the cell (Tardy et al., 2020). Magnesium has also been found to have neuroprotective effects through its role in maintaining ion homeostasis in neurons, preventing excitotoxity and lowering inflammation (Patel et al., 2024). Zinc has also been associated with an antioxidative effect, especially through its downregulation of the production of reactive oxygen species (Tardy et al., 2020). This role aids in reducing cell damage by the reactive oxygen species (ROS). Within the brain, such ROS lead to neuronal death and thus cognitive decline. Such examples thus illustrate the role of minerals in facilitating brain function, and thus the need for their adequate provision through diet.

Conclusion

The link between diet and brain function occurs through various macro- and micronutrients that contribute to different molecules that influence brain activity. Carbohydrates, for instance, provide the main molecule, glucose, that is the source of energy for the brain. Additionally, they affect the availability of some neurotransmitters and their fermentation by microbiota in the gut can provide SCFAs that have neuroprotective effects. For proteins, apart from their role as structural components of enzymes and cells that form the brain, some of the essential amino acids derived from proteins are the precursors for synthesis of neurotransmitters such as serotonin and histamine. Moreover, dietary proteins are involved in the maintenance of the cells of the nervous system and not only involved in replacement of dead cells but also in regulating the deposition of Aβ that is associated with diseases such as AD that manifest in significant cognitive impairments. Dietary fats contribute to brain activity by offering an alternative source of energy in form of ketone bodies, ensuring integrity of neuronal membranes including synaptic membranes, and providing neuroprotective effects associated with PUFAs. Further, their influence on the metabolism of microbiota in formation of SCFAs that have neuroprotective effects also establish them as critical nutrients for brain function. Vitamins and minerals have antioxidant roles that lower inflammation of cells in the brain, with minerals also having other roles related to their incorporation in such proteins as hemoglobin and cytochromes whose activity is essential for oxygen transport and in oxidative respiration. In summary, diet is thus critical to the function of the brain, an appreciation that has informed efforts to use diet to modify the risk of diseases associated with cognitive decline.

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Mean Sleep Midpoint and Cognitive Control in Adolescents: A Seven-Night Wearable Study By Haya El Dabbagh

To what extent does sleep midpoint across seven nights associated with inhibitory control on the Simon task in adolescents aged 16-18?

Abstract

Adolescence is accompanied by a delay in habitual sleep timing that may be relevant to cognitive control. This study examined the extent to which mean sleep midpoint across seven nights was associated with inhibitory control on the Simon task in adolescents aged 16-18. 21 students sampled through convenience sampling from the same school recorded their sleep data using two different wearable devices, Apple Watch and WHOOP, for seven consecutive nights. Each nightly sleep midpoint was then calculated as the halfway clock time between sleep onset and waking; the values were then averaged for each participant to find the mean midpoint value. Participants then completed 10 practice trials followed by the actual 60-trial computer-based Simon task, which calculated the Simon effect. The Simon effect is defined by mean incongruent reaction time minus mean congruent reaction time. A later mean sleep midpoint was associated with a larger Simon effect (Spearman $p = 0.479$, 95% bootstrap Cl [0.045, 0.779], $p = 0.028$). However, Pearson sensitivity analyses were not statistically significant; these findings suggest that later habitual sleep timing may be associated with poorer task performance. However, the following limitations must be noted: the small convenience sampling and the use of two wearable brands, which limit the strength of the conclusions.

Keywords

Sleep midpoint; inhibitory control; Simon task; adolescents; circadian rhythm; wearable actigraphy; social jetlag

1. Introduction

Adolescence is a period of crucial cognitive development during which adequately regulated sleep is vital. Puberty is accompanied by a delay in circadian timing and thus slows the accumulation of sleep drive, increasing adolescents' tendency to fall asleep and wake later (Crowley et al.). This shift conflicts with externally imposed social demands, including early school schedules, therefore resulting in shortened sleep and irregular sleep timing for students globally. This is a pattern commonly described as social jetlag (Wittmann et al.).

In a study of university students, Phillips et al. found that irregular sleep patterns were associated with poorer academic performance. Additionally, more recently, Yang et al. noted that greater social jetlag in adolescents was associated with poorer school performance after matching participants on several demographic variables. These findings suggest that when and how consistently sleep occurs may be relevant to cognition, particularly in a vulnerable age group.

One cognitive process that may be affected by sleep and serves as a strong index of cognition is inhibitory control, which is the ability to suppress actions when they are unlikely to accomplish valuable results (Kang et al.). The Simon task measures this process using responses to relevant stimuli while ignoring their spatial location; responses are typically slower on incongruent trials than on congruent trials, and this reaction time difference is the Simon effect (Cespón et al.). The present study examined whether mean sleep midpoint across seven nights – used as an indicator of habitual circadian timing – is associated with Simon task performance in adolescents aged 16-18. It was hypothesized that a later mean sleep midpoint would be associated with a larger Simon effect.

1.1 Literature Review

Crowley et al. depicted a delay in circadian rhythm during puberty, promoting a later sleep time for adolescents. This becomes an issue for students globally when fixed obligations set by family or school require early wake times, shortening total sleep duration. When this becomes a continuous long-term burden, students begin to accumulate sleep debt. Wittmann et al. termed this difference of external wake times and free days as social jetlag to emphasize the discrepancy between imposed schedules and biological preference for adolescents.

Sleep midpoint is the clock time halfway between sleep onset and waking and is commonly used as a behavioral indicator of sleep timing and chronotype. Although it does not measure endogenous circadian phase (Randler), research comparing chronotype with dim-light melatonin onset has demonstrated that sleep-based chronotype estimates are associated with physiological circadian timing. Thus, supporting the use of sleep midpoint as an indirect circadian indicator (Kitamura et al).

Research increasingly isolates issues with circadian rhythm as factors correlating with reduced cognition. Phillips et al. monitored 61 university students and found that irregular sleep patterns are linked with delayed melatonin timing and, as a result, lower academic performance. Okano et al. similarly stated that objectively measured sleep consistency was associated with academic performance in college students; in a much larger adolescent sample, Yang et al. found that social jetlag in relation to sleep was associated with lower crystallized intelligence.

The Simon task is a psychology experiment used to measure response inhibition and is used as a measure of cognitive control. It is designed to quantify interference created by an irrelevant spatial cue to calculate a difference between congruent and incongruent trials; the congruent trials are when the stimulus location corresponds to the required response side, whereas the incongruent trials are when the stimulus appears on an opposing location. The difference between incongruent and congruent reaction time is interpreted as the Simon effect, as it reflects several cognitive processes including spatial coding, response selection, and cognitive control, making it a valid representative of cognition (Cespón et al.). In a controlled wakefulness protocol, Bratzke et al. found that irregularities with circadian rhythm have effects on overall response performance; although the Simon effect results remained stable, these results suggest that sleep may influence general alertness without necessarily affecting overall cognition.

The type of measurement was also found to affect interpretation of results between sleep and cognitive performance, where self-reported adolescent sleep estimates can differ from wearable device measurements, which can differ from actual polysomnography (Short et al.). This study uses wrist actigraphy as it has shown useful agreement with polysomnography for estimating sleep measurements; however, it must be noted that specificity and device algorithms remain limitations (Marino et al.).

Furthermore, a gap remains in the research involving wearable device-derived sleep data and its effects on cognitive performance using a targeted response task to measure such a vulnerable group of individuals in society being adolescents. The present study aims to address this gap by comparing the midpoint of sleep of adolescents across seven nights with Simon task performance.

2. Methodology

2.1 Experimental design

This study used a correlational design in which sleep was observed under the participants' usual living conditions for seven consecutive nights. After the seven nights, the participants completed a computer-based Simon task programmed by the investigator. The primary variable measured was the midpoint of sleep to measure circadian rhythm. To ensure the reliability and validity of the data collected from the experiment, the following variables were controlled: the same Simon task program, computer, instructions, practice period, and number of trials for all participants.

2.2 Participants

21 students aged 16-18 years were recruited through convenience sampling from the same school. Recruiting the participants from the same single school reduced variation in institutional schedules, as well as homework requirements that may cause the students to sleep later to make submission times. However, it acts as a limitation to generalizing the results of the study to other populations of adolescents. The participants were screened using a questionnaire to remove any students who do not qualify for the study by possessing factors that may influence the data, such as having any sleep disorders, not being of the right age group, not having a wearable sleep device, or not providing personal and parental consent. Once the participants were recruited, they were assigned participant IDs as study identifiers to exclude their names and maintain their anonymity for ethical purposes.

2.3 Materials

Sleep was recorded using consumer wearable devices: Apple Watch and Whoop. The difference in brand of the wearable device must be noted as a limitation because the two manufacturers use different proprietary algorithms for deriving the sleep data. Additionally, the wearable devices are accurate in estimating sleep data when conducting field research; however,

it does not equate to polysomnography, therefore, acts as an additional limitation to the accuracy of the study's sleep data (Marino et al.).

The participant screening questionnaire and the parental consent form were administered through Google Forms. Cognitive performance was assessed using a Simon task programmed by the experimenter and administered on the same computer; this was to eliminate any variances in reaction speed timings between different computers and unofficial Simon tasks.

2.4 Procedure and data collection

Eligible participants received study instructions and were assigned their own participant ID. For the seven consecutive nights of the experiment, each of the participants wore their own wearable device to record their sleep. In the morning, as soon as they woke, they were asked to provide the recorded sleep values to the investigator to be recorded onto the sleep log, where the raw data values were recorded to produce 147 nightly observations.

At the end of the monitoring period, the participants completed the Simon task under standardized instructions. In the Simon task, the instructions were displayed clearly, followed by ten practice trials with feedback, and then the 60 experimental trials were conducted without feedback. The Simon task presented red or blue squares on either the left or right side of the screen. Participants pressed F for red and J for blue while ignoring stimulus position.

2.5 Data processing

The raw entries from the original sleep log for the seven consecutive nights of each participant were kept, providing a record to check if there are any later discrepancies in the data. The timings for the sleep and wake times were then converted to decimal hours to make the mathematical calculations possible. To adjust wake times properly when they crossed midnight to represent a continuous interval, 24 hours were added.

A sleep midpoint was then calculated for every night. If the calculation produced a time greater than 24, it was converted back into normal clock time by taking the value modulo 24. The midpoint calculation might produce 27:00, and a normal clock only goes to 24:00; thus, subtracting 24 would convert the 27:00 back into 3:00. Each participant's seven nightly midpoint values were averaged to produce one mean sleep midpoint. Because all nightly sleep midpoints fell within the same period, none crossing the midnight boundary, the seven midpoint values could be averaged using an ordinary arithmetic mean. A later mean value for each participant represented later habitual sleep timing. Mean sleep midpoint was treated as a behavioral marker representing circadian rhythm rather than a direct physiological measurement of endogenous circadian phase. A worked example of this calculation for two participants is provided in the Appendix (Table 1).

For the Simon task, the Simon effect was calculated using the following formula: mean incongruent reaction time minus mean congruent reaction time. A larger positive value for the Simon effect would indicate a greater interference from the irrelevant stimulus location, thus signaling lower inhibitory control and therefore representing poorer cognition.

2.6 Statistical analysis

The following variables were summarized using their means, standard deviations, minimums, and maximums: participant age, mean sleep midpoint, and Simon-task outcomes. The sleep midpoint was calculated using the formula of sleep onset + a half of the elapsed interval. The Spearman rank order correlation was selected as the primary test for statistical significance, examining whether the participants with later mean sleep midpoints tended to have larger Simon effects and to examine the overall association between sleep midpoint and the Simon effect.

147 nights of raw sleep data were collected for the 21 participants total, although a summary and mean were calculated for the 7 nights of each participant, creating a row containing their mean sleep midpoint across seven nights, their Simon effects, their mean correct reaction time, their overall accuracy, and their wearable device's brand. (Appendix, tables 2 and 3)

Due to the smaller sample size of only 21 participants, a correlation calculated once could be shifted inaccurately towards particular participants' scores; thus, bootstrapping was used to estimate the uncertainty around each Spearman correlation. After the seven nights were averaged, the final statistical dataset contained one row for each participant, each row representing one. Python was used to create a new dataset containing 21 spaces; it then randomly filled each space using a participant from the original dataset. After selecting one participant, the participant remained available for selection again, thus using a combinatorial approach; this allowed the new dataset to include a participant more than once or not at all. The Python model then calculated the Spearman correlation for this new 21-record dataset; this completed one bootstrap repetition. This process was completed 10,000 times; therefore, there were 10,000 slightly different correlation values produced. If the correlations differed greatly, the original result would be seen as uncertain. The Spearman analysis showed a moderate positive association between mean sleep midpoint and Simon effect ($p=0.479$, $p=0.028$). The 95% bootstrap confidence interval ranged from 0.045 to 0.779, indicating that the association was positive but its exact strength was estimated imprecisely. The Pearson analysis also produced a positive correlation ($r=0.326$), but this result was not statistically significant ($p=0.150$); thus, the direction of the relationship was consistent across both methods although its statistical significance was not. Full descriptive statistics, normality tests, and correlation results are shown in the Appendix (Table 4 and Table 5).

Due to the participants wearing two different brand devices, using two separate proprietary algorithms to identify sleep onset, waking, and sleep stages, the device brand could influence the recorded sleep midpoint and thus, the validity of the data; therefore, the device groups were compared to check whether the Apple Watch participants and WHOOP participants had systematically different trends and outcomes. The variables compared were mean sleep midpoint, Simon effect, mean correct reaction time, and overall accuracy. The main analysis examined was the relationship between mean sleep midpoint and Simon effect across both groups. These are exploratory analyses as their results are not used to support the hypothesis, but

rather additional checks to investigate the influence of the difference in brands. The Mann-Whitney U test was used as it compares two independent groups; in this study, the two groups were Apple Watch users and WHOOP users. The test was also deemed appropriate because the groups were small, the variables could not all be assumed to be normally distributed, and the participants in the Apple Watch group were different from the participants in the WHOOP group. The Mann-Whitney test ranked all 21 participants' values from lowest to highest, then analyzed whether one device group generally had higher or lower ranks than the other. The device comparison results for mean sleep midpoint showed that the Apple Watch median was at approximately 03:27; for the WHOOP, it was approximately 03:29; the Mann-Whitney U was 39, and the p value was 0.303, demonstrating a difference of only approximately two minutes, and the difference was not statistically significant. For the Simon effect, the Apple Watch median was 54 ms, with the WHOOP median being 70 ms; the Mann-Whitney U was 29; however, the p value was 0.081, proving the difference between the medians of the Simon effect to be statistically insignificant. For the Mean reaction time, the Apple Watch showed a median of 515 ms, WHOOP users showed a median of 561 ms, U was 30, although the difference was not statistically significant due to the p value being 0.095 ($p>0.05$). Lastly, for overall accuracy, the Apple Watch median was 92.95%, and the WHOOP median was 90.10%; the U was 79, and the p value was 0.082; the difference was not seen as statistically significant. Full results of these comparisons are reported in the Appendix (Table 6).

2.7 Ethics

Participation in the study was voluntary, with participants aged 18 or older providing written informed consent. Due to most of the participants being under the age of 18, they provided written assent whilst their guardians provided written consent. To keep the anonymity of the participants, the subjects were provided with study identifiers to keep the analytical datasets without any names.

3. Results

Across participants, the mean sleep midpoint was 03:42 (SD = 0.99 hours; median = 03:28; range = 02:10-06:03). The mean Simon effect was 65.90 ms (SD = 21.65; median = 60.00; range = 44.00-116.00). Mean correct reaction time was 539.67 ms (SD = 70.26; median = 533.00; range = 436.00-678.00), and mean overall accuracy was 91.65% (SD = 4.05; median = 92.20; range = 83.90-97.40).

3.1 Primary analysis

Mean sleep midpoint was positively associated with the Simon effect (Spearman p =0 .479, 95% bootstrap CI [0.045-0.779], p = 0.028; see fig. 1). Participants with later average sleep midpoints therefore tended to show larger differences between incongruent and congruent reaction time.

3.2 Secondary and sensitivity analyses

Each p-value was above 0.05, thus proving any differences between the groups were seen as statistically insignificant.

The software used to analyze the data was Python; Python 3.12.13 provided the analysis environment, whilst pandas 2.2.2 imported, organized and merged the spreadsheet data, NumPy 2.0.2 performed numerical calculations and generated the bootstrap resamples, and SciPy 1.16.3 calculated the Shapiro-Wilk, Spearman, Pearson and Mann-Whitney U tests.

A later mean sleep midpoint was associated with slower mean correct reaction time (ρ = 0.451, 95% bootstrap CI [0.008, 0.780], p = 0.040, FDR-adjusted q = 0.040) and lower overall accuracy (ρ = -0.471, 95% bootstrap CI [-0.780, -0.034], p = 0.031, q = 0.040).
Pearson sensitivity analyses were weaker and did not reach statistical significance for the Simon effect (r =0.326, p = 0.150), mean correct reaction time (r = 0.369, p = 0.100), or overall accuracy (r = -0.384, p = 0.086). Exploratory device-group comparisons also did not reach statistical significance for mean sleep midpoint or any of the three cognitive outcomes (all p ≥ 0.081).

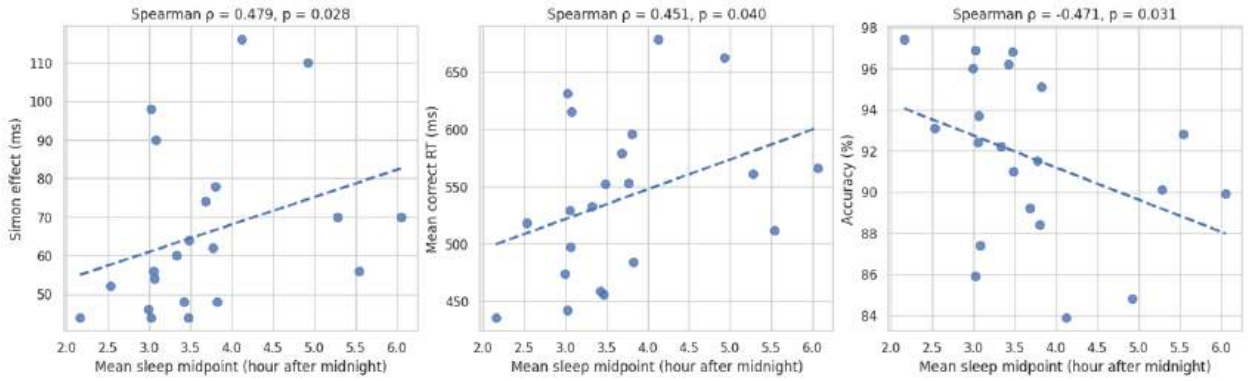


Fig. 1. Associations between mean sleep midpoint (hours after midnight) and cognitive outcomes across all 21 participants. Left: Simon effect in ms, the primary outcome (Spearman ρ = .479, p = .028). Centre: mean correct reaction time in ms (ρ = .451, p=.040). Right: accuracy in percent (ρ = -.471, p = .031). Dashed lines represent lines of best fit; each point represents one participant's seven-night mean.

4. Discussion

This study examined whether mean sleep midpoint across seven nights was associated with inhibitory control performance as a representative measure of cognition in adolescents aged 16-18. The primary result was that later sleep midpoint was associated with a larger Simon effect, which indicates a larger difference between incongruent and congruent reaction times, meaning that these participants were more affected by the irrelevant location of the stimulus and therefore suggesting signs of lower inhibitory control. Later timing was also associated with slower mean correct responses and lower accuracy. These results support the hypothesis that later midpoints would result in lower Simon task performance.

The results follow the same general pattern as previous research connecting sleep timing with academic outcomes. Phillips et al. found that students with more irregular sleep schedules tended to have later melatonin timing, resulting in poorer academic performance. Similarly, Okano et al. associated objectively measured sleep characteristics with academic performance. Yang et al. also reported cognitive associations with social jetlag in adolescents. However, this study differs as it did not measure social jetlag or academic performance directly; rather, it specifically tested mean habitual sleep timing through measuring the sleep midpoints and performance on the Simon response conflict task to represent cognition. Thus, demonstrating the context the previous research provides for the study rather than being a direct replication.

The study found a significant association between sleep timing and the Simon effect, which differs from the results of Bratzke et al., who found that circadian timing affected general response performance although the Simon interference remained seemingly stable. The studies used different methods, however, which may account for the inconsistency between the research; the present investigation observed naturalistic sleep timing in adolescents, whereas Bratzke et al. used a controlled wakefulness protocol.

The results from the present study must be interpreted cautiously, however, seeing as the Spearman correlations reached statistical significance, whereas the Pearson sensitivity analyses did not, as well as the bootstrap confidence intervals had lower bounds close to zero. This analytical sensitivity suggests that apparent relationships may depend on the small sample size. There are several features of the study design that reduce how confidently the results can be interpreted. For example, the sample of 21 participants presents as a limitation due to its small nature, providing less information about the wider adolescent population. Additionally, it reduces statistical power, meaning the study had a lower chance of proving a genuine association. Furthermore, recruiting from one school also presents as a limitation due to students from the same school sharing similar schedules, academic demands, and social environments, which thus limits generalizability of the findings to adolescents in different schools and countries. Next, the two wearable brands used different proprietary algorithms to measure sleep onset, wake time, and sleep stages; therefore, they may have calculated these things differently and provided inaccuracies in the results. It must be noted that device comparisons were conducted to ensure the data could still be used, and no statistically significant device group differences were detected. Lastly, sleep midpoint is calculated from observed sleep onset and wake time. This provides an estimate of habitual circadian timing, although it does not directly measure the biological circadian clock; thus, it must be viewed as an indirect measure of circadian rhythm. Future research should recruit a larger sample from a variety of schools to increase the statistical power as well as allow for generalizability of the results. Additionally, future studies should use one validated wearable device to allow the sleep data estimates to be more consistent across all participants.

5. Conclusion

The research question asked to what extent the sleep midpoint was associated with inhibitory control and response speed in adolescents aged 16-18. A midpoint was calculated for each of the seven nights, then the seven values were averaged to produce one mean sleep midpoint for each participant. In the study, the findings showed that a later mean sleep midpoint was moderately associated with a larger Simon effect. Larger Simon effects indicate lower inhibitory control. The results provide evidence of an association. The associations were significant using Spearman rank correlations but not Pearson correlation analyses. The direction of the relationship was consistent, but whether it reached statistical significance depended on the correlation method used. The results do not prove that later habitual sleep caused poorer performance, as it was not a causal study, and rather it proved an association.

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Appendix

Table 1. Example of nightly sleep midpoint calculations from wearable recorded sleep onset and wake times (illustrative excerpt: participants P001 and P002).

Participant ID	Night #	Device	Wake Time (Clock)	Sleep Onset (Hour)	Wake Clock (Hour)	Adjusted Wake (Hour)	Overnight Interval (Hours)	Sleep Midpoint (Hour)
P001	1	Apple Watch	06:02:00	22.716667	6.033333	30.033333	7.316667	2.375
P001	2	Apple Watch	06:10:00	23	6.166667	30.166667	7.166667	2.583333
P001	3	Apple Watch	06:05:00	23.083333	6.083333	30.083333	7	2.583333
P001	4	Apple Watch	06:10:00	22.833333	6.166667	30.166667	7.333333	2.5
P001	5	Apple Watch	06:00:00	23.166667	6	30	6.833333	2.583333
P001	6	Apple Watch	06:15:00	23	6.25	30.25	7.25	2.625
P001	7	Apple Watch	06:05:00	22.916667	6.083333	30.083333	7.166667	2.5
P002	1	WHOOP	07:15:00	0.45	7.25	7.25	6.8	3.85
P002	2	WHOOP	06:00:00	0	6	6	6	3
P002	3	WHOOP	06:05:00	0.166667	6.083333	6.083333	5.916667	3.125

Table 2. Participant-level mean sleep midpoint across seven nights (N = 21)

Participant ID	N Nights	Mean Sleep Midpoint (Hour)	Mean Sleep Midpoint (Clock)	Age	Device
P001	7	2.535714	02:32	17	Apple Watch
P002	7	3.484524	03:29	17	WHOOP
P003	7	3.684524	03:41	17	Apple Watch
P004	7	3.02381	03:01	17	Apple Watch
P005	7	2.994048	03:00	16	WHOOP
P006	7	3.053571	03:03	16	Apple Watch
P007	7	4.92381	04:55	16	WHOOP
P008	7	3.077381	03:05	17	WHOOP
P009	7	3.821429	03:49	17	Apple Watch
P010	7	5.278571	05:17	18	WHOOP
P011	7	3.469048	03:28	18	Apple Watch
P012	7	3.803571	03:48	17	Apple Watch
P013	7	2.161905	02:10	17	Apple Watch
P014	7	3.025	03:01	17	Apple Watch
P015	7	3.767857	03:46	17	Apple Watch
P016	7	4.122619	04:07	17	WHOOP
P017	7	5.540476	05:32	17	Apple Watch
P018	7	3.42381	03:25	17	Apple Watch
P019	7	6.055952	06:03	18	WHOOP
P020	7	3.063095	03:04	17	WHOOP
P021	7	3.327381	03:20	17	WHOOP

Table 3. Participant-level mean sleep midpoint and Simon task outcomes (N=21)

Participant ID	Age	Device	Mean Sleep Midpoint (Hour)	Mean Sleep Midpoint (Clock)	Mean Correct RT (ms)	Accuracy (%)	Congruent RT (ms)	Incongruent RT (ms)	Simon Effect (ms)
P001	17	Apple Watch	2.535714	02:32	518	93.1	492	544	52
P002	17	WHOOP	3.484524	03:29	552	91	520	584	64
P003	17	Apple Watch	3.684524	03:41	579	89.2	542	616	74
P004	17	Apple Watch	3.02381	03:01	631	85.9	582	680	98
P005	16	WHOOP	2.994048	03:00	474	96	451	497	46
P006	16	Apple Watch	3.053571	03:03	529	92.4	501	557	56
P007	16	WHOOP	4.92381	04:55	662	84.8	607	717	110
P008	17	WHOOP	3.077381	03:05	615	87.4	570	660	90
P009	17	Apple Watch	3.821429	03:49	484	95.1	460	508	48
P010	18	WHOOP	5.278571	05:17	561	90.1	526	596	70
P011	18	Apple Watch	3.469048	03:28	456	96.8	434	478	44
P012	17	Apple Watch	3.803571	03:48	596	88.4	557	635	78
P013	17	Apple Watch	2.161905	02:10	436	97.4	414	458	44
P014	17	Apple Watch	3.025	03:01	442	96.9	420	464	44
P015	17	Apple Watch	3.767857	03:46	553	91.5	522	584	62
P016	17	WHOOP	4.122619	04:07	678	83.9	620	736	116
P017	17	Apple Watch	5.540476	05:32	512	92.8	484	540	56
P018	17	Apple Watch	3.42381	03:25	459	96.2	435	483	48
P019	18	WHOOP	6.055952	06:03	566	89.9	531	601	70
P020	17	WHOOP	3.063095	03:04	497	93.7	470	524	54
P021	17	WHOOP	3.327381	03:20	533	92.2	503	563	60

Table 4. Descriptive statistics and Shapiro-Wilk normality tests for participant-level variables (N=21).

Variable	n	Mean	Median	Minimum	Maximum	Shapiro-Wilk W	Shapiro-Wilk p
Age	21	17	17	16	18	0.7236	0.0001
Mean Sleep Midpoint (hour)	21	3.6971	3.469	2.1619	6.056	0.8964	0.0298
Mean Correct RT (ms)	21	539.6667	533	436	678	0.9661	0.6451
Accuracy (%)	21	91.6524	92.2	83.9	97.4	0.9562	0.4438
Congruent RT (ms)	21	506.7143	503	414	620	0.9712	0.7584
Incongruent RT (ms)	21	572.619	563	458	736	0.9596	0.5086
Simon Effect (ms)	21	65.9048	60	44	116	0.8708	0.0099

Table 5. Correlations between mean sleep midpoint and Simon task outcomes (N=21).

Analysis Family	Outcome	Spearman ρ	Bootstrap 95% CI Lower	Bootstrap 95% CI Upper	Bootstrap Resamples	Pearson r	Pearson p	FDR-Adjusted q
Primary	Simon Effect (ms)	0.479	0.0445	0.779	10000	0.3258	0.1495	—
Secondary	Mean Correct RT (ms)	0.4506	0.0078	0.7804	10000	0.3692	0.0995	0.0403
Secondary	Accuracy (%)	-0.4714	-0.7797	-0.0341	10000	-0.3838	0.0859	0.0403

Table 6. Exploratory Mann-Whitney U comparisons of Apple Watch and WHOOP participants (N=21)

Variable	Apple Watch n	Apple Watch Median	WHOOP Median	Mann-Whitney U	p Value
Mean Sleep Midpoint (hour)	12	3.4464	3.4845	39	0.3028
Simon Effect (ms)	12	54	70	29	0.081
Mean Correct RT (ms)	12	515	561	30	0.0949
Accuracy (%)	12	92.95	90.1	79	0.0817

Stressors of Long-Term Hospitalization in Pediatric Patients and Strategies for Mitigation
By Peri Rosenberg

Abstract

This systematic review examines what stressors pediatric patients undergo during long-term hospital stays and what interventions hospitals should implement to eliminate these stressors. There is a gap in how pediatric care addresses mental health during hospitalization. Prior research identifies stressors and mitigation strategies, but lacks continuity in examining how these stressors are managed and monitored over time to influence mental health outcomes. Articles were selected from academic databases and filtered for age, length of hospital stay, and medical condition. A thematic analysis was performed to identify common themes of stressors and interventions across studies. The results show that consistent parental involvement decreases anxiety and supports emotional security. Recognizable personal items from home help maintain a sense of familiarity and comfort. Additionally, maintaining a routine provides children with a sense of predictability and control. These small changes to a patient's routine help eliminate their stressors and should be implemented in every children's hospital.

Introduction

Seven percent of hospitalized children develop a mental health issue after they are discharged from the hospital.[1] The primary responsibility of hospital workers is to ensure that patients are safe and healthy. In trying to achieve these goals, hospital workers inadvertently create a stressful environment for patients through the food, sterile smell, lighting, and intense surroundings. Previous research confirms that, until the middle of the last century, hospital workers solely focused on curing children's illnesses and did not address the rest of the children's needs.[2]

While previous research has identified the stressors associated with hospitalization and strategies to reduce them, the literature remains fragmented. Most research examines individual stressors or interventions in isolation, without exploring how these factors interact over time. Additionally, much of the available research is more than a decade old, limiting its relevance to modern pediatric hospitals where evolving technologies and care environments may bring new sources of stress. This research will address these limitations by synthesizing current evidence to identify how hospitals and caregivers can better support patients' emotional well-being, explain what stressors pediatric patients undergo during long-term hospital stays, and how hospitals can eliminate these stressors. By integrating recent research, this review provides an updated understanding of effective strategies for supporting pediatric patients during hospitalization to minimize the risk of long-term adverse mental health outcomes. The findings aim to guide doctors, nurses, family members, and hospital workers to create an environment where young patients feel more at ease during hospitalization.

Methods

A systematic search was conducted to find relevant literature examining the relationship between mental health and long-term hospitalization in pediatric populations. The research studied includes children aged 5-15 with chronic diseases such as diabetes, asthma, sickle cell disease, cystic fibrosis, hematological or oncological disorders, and various types of transplants. Both male and female children were sampled, and the length of their hospital stays varied greatly. These studies were collected from academic databases. Keywords used in the searches included: “long-term hospitalization,” “pediatric,” “mental health,” “stressors,” “social isolation,” and “therapy.” Articles were initially screened based on title and abstract to determine relevance to the research question, followed by full-text review for eligibility. Studies were included if they focused on pediatric patients, addressed mental or emotional outcomes associated with hospitalization, and applied to extended hospital stays. Studies that did not directly address these outcomes, focused on adult populations, or lacked relevance to prolonged hospitalization were excluded. Data were extracted from each selected study with particular emphasis placed on the results and discussion sections. A thematic analysis was then conducted, where extracted information was organized and reviewed to identify recurring themes. Once themes were identified, the original manuscripts were reexamined to ensure that each theme was supported by consistent evidence. The quality and reliability of the included studies were assessed by comparing the findings across multiple peer-reviewed articles addressing similar topics.

Results

I. Environmental and Emotional Stressors

Across the studies analyzed, the most consistently reported stressor for hospitalized pediatric patients was unfamiliarity with the environment. In the hospital, children are removed from their normal routines, physical environment, and social networks. This intensifies anxiety–especially when they are already coping with illness.[3] The clinical intensity of a hospital adds to the children's stress. Children report that the hospital environment has noise levels eight times higher than recommended fostering a frightening environment for the patients.[4] Several studies identified procedural stressors, including: “needles, nasogastric tubes, dressings, lumbar punctures, bone marrow tests, chemotherapy, dying, other children dying, and not being around their friends.”[5] These stressors include both physical pain and emotional hardship. For children undergoing long-term hospitalization, repeated exposures throughout their stay can compound over time, creating higher stress levels and difficulty with emotional recovery. Charles Schleien, senior vice president of pediatric services at Northwell Hospital, argues that children are not given the mental treatment that they deserve because of stigmas surrounding mental health. [6]

Family dynamics also significantly impact children’s stress levels. Children often rely on their parents for emotional support. Because of this, parental anxiety can directly affect pediatric coping and adjustment. One study found that the strongest indication of behavior shifts amongst

children were the impact of the illness on families.[7] This suggests that these relatives will not be able to uplift the children as they too experience stressors. Therefore, children's distress levels reflect those of their parents as they often look to their parents for strength and comfort. Overall, pediatric stress during long-term hospitalization comes from many factors that stem from the unfamiliarity of the hospital environment, repeated medical procedures, social isolation, and parental reactions.

II. Reducing Stress Through Parent Involvement

Beyond environmental changes, parental involvement was identified as one of the most crucial factors to their child's mental health during hospitalization. Multiple studies showed that parents play a central role in helping children maintain emotional stability and a sense of identity. For example, Manning et al. showed that parents were instrumental in directing the participants' aspirations to retain their former selves.[9] This highlights how parents help children preserve continuity between their life before hospitalization and during treatment. A study that tested the pretest and posttest scores of parents' anxiety levels to hospitalized children found that, when parents participate in caring for their hospitalized children, the anxiety is reduced in both parents and children.[10]

Parental emotional regulation is critical in reducing their children's stress levels. Children look to their parents to model their emotional responses. High parental stress can cause both parties' stress to increase. Children focus on their parents' reactions rather than their words.[11] Therefore, calm parental demeanor may be more effective than repeated verbal reassurance alone. Further, research suggests that while having both parents in the hospital significantly reduces a child's stress, children often only look to their mothers for cues on how to act. One study observed that when a nurse had deeper conversations with a patient and their families, the mother's stress levels declined.[12] Reductions in maternal stress, therefore, positively influence the child's emotional well-being. Thus, interventions aimed at reducing pediatric stress should also include support systems for parents.

III. Reducing Stress Through a "Home-Like" Environment

In response to these stressors, multiple studies found environmental modifications such as having less harsh lighting made by hospital clinical care teams and administration to be effective interventions. Many hospitals across the country are implementing these changes, for example the President and CEO of Northwell, Michael J Dowling, has committed 500 million dollars to pediatric mental health.[6] By making the hospital feel more like home for patients, the atmosphere reduced pediatric anxiety and improved emotional well-being. Children associate home with safety and comfort; therefore, by including elements of normal life in the hospital setting, they can counteract the stress caused by unfamiliarity. Children's favorite parts of their hospital experience were "[m]ovies to pass time, homemade cooking, wearing hats, listening to music, [and] massages."[5] These activities reflect ordinary, comforting experiences typically associated with home. By recreating elements of home and normalcy, these activities help

hospitalized children momentarily forget their illness and feel more like themselves, and in turn, less stressed.

Additionally, the presence of routines and “habitual activities” emerged as an important source of comfort.[8] Maintaining consistent schedules and encouraging children to establish habits similar to those they already have at home supports a sense of predictability and control. One source noted that “the focus on normality” motivated participants to assume roles and routines they had undertaken before becoming critically ill.[9] One of these routines is school. Schooling inside of hospitals allows children to have a routine as well as , having interactions with teachers and other students.[2] Establishing these routines allows patients to feel more like themselves and temporarily ignore the harsh hospital environment. These findings indicate that environmental familiarity and the creation of normalcy are powerful tools for mitigating hospital-related distress.

Discussion

The findings of this study help answer the gap in existing knowledge and contribute to how hospital care teams and caregivers should approach children undergoing long-term hospitalizations to reduce the number of pediatric patients who develop mental health challenges as they mature. These mental challenges include acute stress, post-traumatic stress disorder, anxiety, and depression.[1] The biggest stressor for patients was being in an unfamiliar hospital environment. Reminders of home and routines were found to decrease these stressors. To further improve patient mental health outcomes, families should bring home-cooked meals, toys, stuffed animals, and games that remind the patient of home. Furthermore, hospital workers should strive to implement routines in the patient's daily life in order to decrease distress. This may include time for patients to leave their bedrooms, get outside, and interact with other children.

Additionally, patient stress was shown to be directly correlated with parental stress. Because of this, reducing parental stress is crucial for reducing patient stress. For example, hospital workers should aim to relax both the patient and their parents while delivering information about procedures and results.

There were several limitations to this study. The broad age ranges and variability in lengths of hospitalizations limits this research's external validity in its applications to diverse populations. Generalizations were made to account for this heterogeneity, which may limit the generalizability of these findings to populations with different characteristics. The research was also limited by attrition bias, making it difficult to determine the effect on patients and whether the strategies were effective for them later in life.

Due to advancements in modern medicine, more children are surviving long-term hospitalization and experiencing long-term effects, making this research more crucial than ever. By implementing these strategies, hospitals can help ensure that children leave emotionally supported and less likely to develop future mental health challenges.

Acknowledgements

As a lifelong cardiac patient, all of my trips to the hospital have caused a lot of both physical and mental hardships. I wanted to find a solution to the stressors caused in the hospital in order to eliminate many of the worries that patients such as myself feel.

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The Degradation of the Nutritional Quality of Food in America By Henry Allen

Introduction

According to the Centers for Disease Control, the average American receives about 55% of their calories from ultra-processed, additive-rich foods (CDC). In the United States, corporate lobbying and regulatory limitations have contributed to growing public health challenges associated with diet-related diseases. In contrast, the European Union applies stricter oversight to the use of harmful additives, the production of high-risk sweeteners, and the diets of its citizens. The nutritional quality of the American food system has degraded, undermining public health largely as a result of corporate influence and regulatory limitations.

Changing Dietary Patterns in the United States

Americans have consumed more processed and additive-rich foods, resulting in an increased calorie intake and an overall decrease in nutrition. The Economic Research Service, a United States government organization, highlights the 15% increase in the average American calorie intake from 1977 to 2018 (from ~1,807 to ~2,093 calories per day) (Economic Research Service). The additives involved in making such addictive and palatable meals create more calorie-dense foods, resulting in a decrease in overall nutrition. *While the average calories continue to rise,* the National Institute of Health recalls that the percentage of daily energy consumed from home‑food sources declined by 24.5% for men and 23.9% for women between 1965–66 and 2007–08 (NIH). The decrease in more nutritious home-cooked meals has fueled a dependence on more calorie-dense and processed store-bought or restaurant-prepared foods. American government subsidies have made HFCS an increasingly inexpensive alternative to natural sugar. This affordability has led companies to increase the quantity of the additive in their products to enhance taste despite the negative effects on consumer health. The extensive use of HFCS by profit-driven companies is associated with obesity trends and the general degradation of American dietary health, alongside other contributing factors. The FDA's outdated and poorly enforced food additive regulations allow thousands of chemicals into the U.S. food supply, many of which are linked to obesity, cancer, and other chronic diseases (Nutrition Studies). Over time, this has resulted in numerous additives, some of which have been associated with increased health risks in the average diet, resulting in the downward spiral of general American nutrition.

Regulatory Differences Between the United States and the European Union

While the American nutrition system has declined, governments overseas have demonstrated how the use of stricter regulations results in a more nourishing diet. According to the American National Standards Institute, "The U.S. generally allows a wider variety of additives. For example, potassium bromate is still permitted in the U.S., even though it's banned in the EU due to cancer risk concerns"(ANSI) while "The EU has banned or heavily restricted the use of several additives that are allowed in the US"(ANSI). As seen in the contrasting legislation between cancer-causing additives, including potassium bromate, it is notable that the

FDA often fails to prevent hazardous additives from being put on American shelves; the European Food Safety Authority (EFSA) implements more protective policies. Yuka, an organization with a database covering the nutrition details of over 3 million food items internationally, specified in a study, "additives are everywhere in American products. Their use is often poorly regulated, with a much looser regulatory framework than in Europe … Worse still, some additives that are banned in many countries remain authorized on the U.S. market"(Yuka). For instance, bread sold in America has nearly 3 times as many additives and 2 times as many high-risk additives as bread found in the EU (Yuka). On the other hand, the more robust mandates of the EFSA have resulted in significantly fewer additives in food, as seen through the comparison in the extent of additive use in bread. The European Union summarizes that its policies aim to prevent the overproduction of additives such as HFCS through reference pricing (European Union). While the United States routinely fails to use policies to limit the overproduction of nutritionally harmful sweetening agents, the EU has enacted multiple provisions that have effectively decreased the degree to which sweeteners are included in consumables under EU authority. The European Union's use of impactful legislation has led to less sweetened food and lower baseline sugar consumption. *As the American government continues to allow these additives,* the National Library of Public Medicine concludes that the use of additives in foods results in serious long-term health effects. These include "increasing risks of mental health disorders, attention deficit hyperactivity disorder, cardiovascular disease, metabolic syndrome, and potential carcinogenic effects"(Warner). Additionally, additives, which are particularly attractive to children, are "a ticking time bomb for adult obesity, metabolic syndrome, cardiovascular diseases, mental health disorders and cancers"(Warner). Additives, which have become extremely common in American foods due to weak policies made by the FDA, not only have immediate effects on adults, including an enhanced susceptibility to mental disorders, lower attention spans, cardiovascular disease, and a variety of cancers, but also contribute to the same consequences with the addition of adult obesity. In the European Union, the health of its citizens is greatly considered when making nutrition-related policies, while the United States is more lenient, resulting in healthier diets in the EU, while Americans are subjected to serious negative health effects.

Agricultural Policy and Corporate Influence

The degradation of the American diet is significantly affected by agricultural policies, monetarily driven policy makers, and corporations. U.S. government subsidies have driven the price of corn down by 27%, leading to High Fructose Corn Syrup (HFCS) becoming significantly cheaper than traditional sugar (LISD). As a result of U.S. agricultural policies, HFCS has been added excessively and inexpensively into the products of large companies seeking to create more addictive foods regardless of the consequences on consumer health. The American Heart Association explains that Americans ingest nearly 3 times as much added sugar as recommended (Poti). U.S. agricultural legislation leads to this as it permits the outrageous integration in the average American diet (Poti). Although obesity or other dietary diseases are

typically blamed on the individual, politics has a disproportionately large amount of control over its citizens' diets. The consumption of almost 3 times the recommended daily amount of added sugar is not a result of consumer neglect but government disregard. In an article for the Union of Concerned Scientists, Omanjana Goswami and Karen Stillerman claimed that “Giant corporations, along with the industry associations that represent and align with them, dominate the US food and agriculture system. Together, they have hijacked federal farm policy for their profit”(Stillerman and Goswami). What drives the U.S. government to continue legislating policies that dismiss the health of American citizens is the relentless lobbying enacted by profit-driven corporations. As described by Frank Dixon in a study published by the Center of Nutrition, “Many of the additives used in the US are banned in other countries … US food products contain thousands of additives… They also degrade human health or have the potential to do so”(Dixon). When government policies fail to regulate what enters the food environment, serious human health issues face unnecessary ramifications. While the EU demonstrates ideal policies regarding food, organizations within the U.S. are guided by profit, resulting in significant residual effects on health.

Conclusion

Historically, the United States has shifted to a more lenient nutrition regulatory framework, influenced by lobbying and other economic incentives, encouraging higher frequencies of ultra-processed foods to enter the market, resulting in preventable diet-related diseases. Conversely, the European Union operates a more precautionary system, keeping surplus additives and sweeteners from entering the consumer market, protecting long-term health. Ultimately, the differences in the nutritional policy-making of the United States and the European Union represent both a reason for the degradation of American health but also an example to follow. Each year, thousands of people die prematurely from diet-related illnesses by diet-induced conditions despite governments like the European Union already demonstrating how the use of policies can save thousands of people each year.

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Overdiagnosis in Mental Problems and Its Impact By Joshua Lin

This review examines the growing problem of overdiagnosis in mental health and its consequences for individuals and society. As mental health awareness increased, more people have sought treatment and attention for psychological distress. Although this has improved access to care and reduced stigma, it has also contributed to the pathologization of daily occurrences through the broader definitions of mental illness. This paper explores the diagnostic expansion in the DSM-5 and DSM-5-TR, particularly how it may contribute to overdiagnosis in conditions such as PTSD, ADHD, bipolar disorder, and prolonged grief disorder. It then evaluates the medical, psychological, and social consequences of overdiagnosis, including unnecessary treatment, stigma, distorted self-perception, and the tendency to overlook environmental and structural causes of distress. Ultimately, the paper argues that psychiatric diagnosis should balance the need to recognize legitimate suffering with greater caution toward unnecessarily expanding the boundaries of mental disorder.

Introduction

In the modern world today, there has been a dramatic increase in efforts to spread mental health awareness and recognition. For example, campaigns such as “Take the Moment” launched by the National Alliance on Mental Illness that encourage open discussion on stress and anxiety (NAMI,2024), the World Mental health Day campaigns hosted by the World Health Organization(Salamon, 2025) , “Live Your PosSCZible” campaign by celebrity Taye Diggs that supports awareness for Schizophrenia related disorders(Live your PosSCZible, 2025), and self evidently, the increased presence of mental health information on social media platforms like Tiktok, Instagram, and Twitter. These recent developments have decreased the stigma surrounding mental illnesses out of understanding and care of the diagnosed, as well as the recognition of previously unrecognized mental disorders. This type of close exposure to the world of mental health encouraged individuals to seek out psychiatric assistance and treatments, acknowledging distress that may have been previously ignored or stigmatized.

These developments have indeed improved the recognition of psychological suffering. However, this has also contributed to an increasingly expansive concept of mental disorders that pathologize normal distress. As a result, overdiagnosis has emerged as a huge issue in a time of increased awareness and visibility.

Overdiagnosis is the diagnosis of a disease or medical condition that is technically present, but will not significantly influence the individual in terms of health, daily functioning, or treatment if left undetected(Singh et al, 2018). Overdiagnosis is not only prevalent, but also harmful to society: individuals commonly experience unnecessary medical treatment, and harmful psychological and social consequences that distort how individuals view themselves and are viewed by society. In this essay, I aim to establish the magnitude of the problem of overdiagnosis, mainly through explaining the factors that have contributed to the growing prevalence of overdiagnosis and its harmful effects on the individual and the society.

DSM-5 and Concept Creep

Increases in the diagnostic criteria has led to higher rates of overdiagnosis. The society has experienced an increase in these areas through the modifications and expansions of the Diagnostic and Statistical Manual of Mental Disorders(DSM), with its latest version being the DSM-5-TR. New editions of the DSM 5 have been updated and changed for a variety of reasons: increased emphasis on preventative measures, as small symptoms must be detected early to reduce risk of severe illnesses; the filling of diagnostic gaps, as there are various issues that are not covered by specific diagnostic criterias; and the desire to solve different diagnostic problems(Boysen et al, 2014). However, not all modifications to the DSM-5 reflect diagnostic expansion. Diagnostic tightening is also commonly implemented by the American Psychological Association to address numerous issues.

To prove that there is an overall net increase in diagnostic criteria in the changes of the DSM, research conducted by Guy A. Boysen and Ashley Ebersole highlighted that in 83 disorders analyzed between the DSM IV-TR and the DSM-5, 32 showed no net change, 21 showed a net change towards exclusivity, and 30 showed a net change to inclusivity(Boysen et al, 2014).

This net movement towards inclusivity in the latest editions of the DSM are caused by what psychologists refer to as Concept Creep. Concept Creep is the gradual expansion of diagnostic definitions, which seeks to include a wider variety of symptoms, experiences, and behaviors that are perceived as non-pathological or innocuous(Boysen et al, 2014). Psychiatric criterias are naturally exclusive; these criterias are operational definitions that help guide healthcare professionals to make distinctions between an actual condition and subjective, individualized experiences of stress or anger.

However, as individuals gain more knowledge and confidence in their ability to detect and respond to mental health problems through campaigns and efforts to spread awareness, they begin to seek medical assistance and perspective on varying levels of symptom severity. Consequently, healthcare professionals experience questions and concerns from many individuals whose symptoms just fall beyond the diagnostic boundaries, or those who face different and new types of distress that were not considered clinically pathological(Boysen et al, 2014). These instances are referred to as “false negatives”, where individuals experience distress and partial symptoms without fully qualifying for a diagnosis(Boysen et al, 2014). These “false negatives” are perceived as problematic in the field of psychiatry because it has the potential to exclude individuals from recognition, treatment, and research. In this way, diagnostic criteria in the DSM-5 and the overall concept of mental disorders are expanded in order to include the increasing variability of symptoms and individual experiences, ultimately creating what psychologists describe as concept creep.

Concept creep has been applied throughout numerous categories of mental disorders. One example of such expansion is Post Traumatic Stress Disorder(PTSD). Originally, PTSD had strict diagnostic boundaries. The earlier edition DSM-III PTSD Criterion A, which defines the requirements for the disorder, emphasized that there had to be a sufficient, harmful stressor that

is directly experienced by individuals(Marx et al., 2023). However, revisions of the DSM-5 allowed the diagnosis of individuals who experienced these symptoms indirectly. This opened up the definition of the stressor to beyond its initial boundaries, changing the originally objective requirements to a more subjective and individualized interpretation of distress and discomfort(Pai et al, 2017). As a result, the prevalence of people reporting trauma increased by 69%(Boysen et al, 2014).

Additionally, the DSM-5-TR included new disorders such as the Prolonged Grief Disorder, which is a psychiatric condition characterized by persistent grief that interferes with an individual's daily life(First et al, 2022). This expands the scope and symptoms of mental disorders by incorporating a universal human experience— intense grief after traumatic and stimulating events— into an official guideline for diagnosing mental health disorders.

Similar patterns of diagnostic expansion can be observed in the Attention Deficit/Hyperactivity Disorder(ADHD). For example, out of 20 studies that examined diagnostic patterns between 2 or more criterias, 18 of the studies showcased a simultaneous increase in potential cases along with the broadening of criteria(Kazda et al, 2021). These increasing ADHD diagnostic rates foster empowerment for individuals looking for a biomedical explanation for their problems, creating a sense of legitimacy and understanding. Yet, many of these studies also reported how increased diagnosis enhanced prejudice and discrimination(Kazda et al, 2021).This introduces how overdiagnosis can create broader psychological and social consequences by influencing how individuals and society perceive themselves and their varying levels of distress.

Implications of Overdiagnosis

Many consequences result from overdiagnosis. This issue is evident in the diagnosis of Bipolar Disorder, a mental condition characterized by shifts in mood and energy levels. Following the formal recognition of the Bipolar 2 Disorder in 1994, researchers introduced the idea of "subthreshold" and "subclinical" Bipolar Disorder for individuals outside the diagnostic boundaries of the disorder, which dramatically increased the prevalence of diagnosable individuals from approximately 1.6% to 24.2% between 1994 and 2007(Doyen, 2021). Additionally, In 2008 only 33% of patients diagnosed with Bipolar Disorder actually met clinical conditions(Doyen, 2021). The most commonly diagnosed communities are the marginalized; those with low social support, low socioeconomic status, and a history of traumatic experiences(Doyen, 2021).

Individuals diagnosed with Bipolar Disorder are commonly prescribed with Lithium, a mood stabilizer(Mayo Clinic, 2026). When Lithium is taken without any severe dysfunctions or abnormalities in an individual's daily mood and behavior, it can increase hypothyroidism and chronic disease, which ultimately contributes to memory loss and decreased executive functioning(Doyen,2021). This demonstrates how the expansive concept of Bipolar Disorder, along with numerous other illnesses, can expose individuals to unnecessary psychiatric treatment and harmful medical consequences, even when their distress may stem from environmental or situational factors rather than biological disruptions in the brain and the mind.

When doctors diagnose individuals with Bipolar Disorder, those people are immediately labeled as biologically ill. Although their symptoms may align with biological problems and impaired functions, putting this label on them ultimately masks oppressive social conditions; instead of recognizing these emotional struggles as normal responses to environmental hardship, this approach increasingly frames this issue through a biomedical lens that interprets distress as evidence of internal psychiatric dysfunction(Doyen, 2021). By viewing distress primarily as a biological problem, psychiatric diagnoses can divert the attention from larger systemic, structural causes such as trauma, poverty, and social inequality, which reinforces the idea that the problem lies within the individual rather than within the conditions shaping their experience. This causes individuals to receive unnecessary treatment that can be both harmful towards their mental health and dismissive of the environmental and social roots of their suffering.

Furthermore, overdiagnosis can actually increase real psychological suffering through distorting the perception of distress in society, creating a cycle that may indirectly inflict more psychological suffering. As noted previously, awareness efforts allowed society to emphasize milder psychological problems as mental health problems that require labelling and reporting. This overinterpretation of psychological disorders can actually create new mental problems and increase the severity of existing ones(Foulkes & Andrews, 2023). This can be described through Ian Hacking's Looping Effect, which explains how psychiatric classifications can reinforce types of behavior and perception that aligns with the illness(Gillespie & Wagoner , 2025) . Once individuals are diagnosed, they may change their behavior and self-concept to align with the diagnosis they have been given, these include social isolation and the distortion of emotions and symptoms. These behaviors reinforce the original diagnosis, where healthcare professionals will take note of these observations and make edits to their classifications, ultimately creating a "loop" where individuals increasingly behave and perceive themselves in a way that aligns with their originally labeled psychiatric categories(Foulkes & Andrews, 2023).

This demonstrates a self fulfilling prophecy: individuals will start to unconsciously think, feel, or act in ways that confirm their diagnosis. As a result, rather than classifying psychological problems, these labels distort interpretations of identity and emotion while reinforcing behaviors that unintentionally intensify distress.

In this case, people lose their ability to interpret themselves and their emotions through broader social, environmental or personal contexts, contributing to a "hermeneutical injustice" where individuals are unable to evaluate their given situation due to the lack of adequate explanation or care from society(Nielsen, 2025) . This results in confusion, isolation, and self blame(Nielsen, 2025). Overdiagnosis can actively influence how individuals interpret their experiences and emotions, creating a cycle of injustice and pathologization that perpetuates the experience of chronic mental illness.

Inherent subjectivity of psychological suffering

Through this paper, I have argued that Overdiagnosis is an increasingly significant issue in a time of expanding mental health awareness and recognition by providing its causes and deep

implications on our society. However, this issue cannot be resolved with absolute certainty. Emotional suffering is deeply subjective; emotions such as fear, anxiety, and stress manifest differently across individuals depending on their history of trauma, the environment they live or grew up in, and personality. Therefore, it is difficult to establish universal psychological boundaries or thresholds that can perfectly distinguish between “normal distress” and “clinically significant, pathological distress”.

Although signs of illnesses can be easily evaluated from a third person perspective, because these experiences are very much personal and individualized, outsiders cannot objectively measure or determine the legitimacy of another person’s emotional suffering. This limits our ability to completely solidify the presence of overdiagnosis in the field of psychiatry.

In retrospect, psychiatric frameworks and diagnostic systems should be improved to prevent the increasingly expansive concept of mental illness and the deep implications of overdiagnosis, while still protecting and respecting the personal and legitimate experiences of those who report psychological distress.

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Circadian Phase Alignment at CAR T-Cell Infusion and Clinical Outcomes in Large B-Cell Lymphoma By Jennifer Duan and Dr. Yan Li

Abstract

Large B-cell lymphoma (LBCL) frequently relapses after chemotherapy and is addressed with CAR T-cell therapy. Some patients experience early relapse or severe toxicity like immune effector cell-associated neurotoxicity syndrome (ICANS) or cytokine release syndrome (CRS). Patients' immune systems are controlled by the body's internal clock, an underappreciated parameter that could influence CAR T-cell efficacy and toxicity. This project investigates whether the problem arises from using "clock time" as a surrogate for "biological time" and develops a "phase alignment hypothesis." The hypothesis proposes that effects could be determined by the relative phase between the time of treatment and the circadian state of the patient, the CAR T-cell product, and the preceding treatment. All three multicenter studies assessing infusion time and outcomes (2,351 patients, 15 centers), as well as experimental studies on circadian control of lymphocyte migration and antigen presentation, were evaluated. Two studies associated later infusion with poorer outcomes, whereas a third found no independent association after adjustment. In one 1,052-patient cohort, each hour later was associated with an 11% higher hazard; one-year progression-free survival was 51.4% before noon versus 35.2% after noon. A separate 715-patient cohort linked later infusion with greater mortality and severe ICANS, but not severe CRS. Crucially, circadian phase was not directly assessed. Clock time served as an imperfect proxy: patients treated at the same hour may be in different biological states. Future work could directly measure circadian phase and evaluate efficacy and neurotoxicity as distinct timing outcomes.

Keywords: CAR T-cell therapy; circadian rhythm; chronotherapy; large B-cell lymphoma; ICANS; cellular kinetics

Introduction

Unlike traditional medicines, CAR T-cell treatments not only affect the initially infused number of cells but also significantly alter the patient's condition over time, depending on the ability of cells to stay active, recognize antigen, navigate to tissue with inflammation, proliferate, and function. It has been established in the case of LBCL that the kinetics of early expansion and the profile of the cell product influence treatment effectiveness [1-7]. The importance of these factors has shifted focus beyond mere dose evaluation to investigate conditions that enable the first encounter between cells and the patient's system to effectively initiate therapeutic activity. Several physiological parameters vary throughout the course of the day. Among them, the circadian system regulates leukocyte recirculation, endothelial cell adhesiveness, antigen processing, hormone levels, and inflammatory response. The body clocks in lymphocytes themselves affect their migration to lymph glands [8].

In addition, dendritic-cell migration is also regulated rhythmically and chronobiologically, and this influences both their antigen processing and T-cell-stimulating capacity [9, 10].

Furthermore, clinical trials have demonstrated that the biological clocks of malignant cells or normal counterparts regulate CD8+ T-cell infiltration into tumors, enhance antitumor activity in CD8+ T-cell therapies, and contribute to tumor growth [11, 12]. Evidence has also emerged from other areas of immunotherapy suggesting that optimizing treatment timing based on chronobiology can be beneficial, while direct clinical data for CAR T cells are relatively new. Early experimental evidence suggests that timing matters; even brief time intervals between administration and the appearance of cellular activity may have lasting consequences [11, 12]. Time-of-day associations have been reported in studies evaluating immunomodulatory therapy such as checkpoint inhibitors [13, 14] or vaccines [15, 16].

Similar associations have been reported for stem-cell transplantation (HSCT) [17]. Therefore, the results of existing clinical studies were revisited and analyzed to determine how best to address and integrate basic and experimental data into clinical decision-making and future trial design for CAR T-cell-based immunotherapy.

The study of Lyons et al. on a cohort of 715 patients from two U.S. centers indicated that later infusion timing was associated with increased mortality and neurotoxicity due to immune effector cells [18].

Luan et al. demonstrated that, in a cohort of 1,052 adult patients, infusion times later than noon compared to those earlier than noon were associated with significant differences in terms of both progression-free survival (25%) and cellular functions (15% reduction in CAR T-cell expansion), as measured on day 7 postinfusion [19]. However, an important study carried out by the team of Dowling et al. at six Australian centers found no markedly significant association between day or night administration and improved response [20]. Thus, the lack of unanimity among those three independent investigations suggests that establishing a 'morning-day-night-time' rule may be premature without taking additional and coordinated steps.

However, in the three available human studies, none included objective assessments of time relevant to the physiological rhythms of individual patients. For the majority of the investigated subjects, a measured time point was used: 11:00 a.m. Treatment was administered to patients who may have very different chronotypes, sleep-wake cycles, daylight exposure, and previous histories of hospitalization; these, in turn, could be influenced by nocturnal visits to hospital, concomitant steroid intake, fever, or use of lymphodepleting agents.

Such events can readily disrupt and desynchronize normal body rhythmicity. Moreover, the timeline related to the CAR T product, i.e., the cell collection, ex vivo amplification, freeze-up, transportation, thawing up, and the time until administration, is a separate factor that has been totally overlooked. Hence, we conducted an in-depth review of the three clinical cohort studies along with experimental data of circadian biology to clarify what could explain, and potentially mediate, time-of-day effects on outcomes. We discuss two major points: First, whether we have a conclusive clinical body of evidence on time-of-day effects that

would warrant a clinical recommendation. Second, whether we could propose reasonable measurements that would allow distinguishing true biological effects of timing from other determinants.

Here, we put forward phase alignment of the timing of therapy, the patient's biological clock, and environmental conditions as a hypothesis to explain these contradictory results.

Methods

Literature search

We performed a comprehensive literature search focusing on studies exploring the time-of-day effects of CAR T-cell infusions and relevant experimental literature describing the mechanistic role of circadian control on T-cell immunity. The literature search was last updated on Aug. 3, 2026.

We systematically searched PubMed/MEDLINE, PubMed Central, journal publisher-provided content (e.g., journals online, SpringerLink, ScienceDirect, Wiley Online Library), Crossref-indexed records, as well as references included in retrieved publications. Keywords used included: 'CAR T', 'chimeric antigen receptor', 'circadian', 'diurnal', 'time of day', 'infusion timing', 'chronotherapy', 'sunrise', 'expansion', 'ICANS', and 'large B-cell lymphoma'. Secondary keyword strings used 'lymphocyte circadian trafficking', 'dendritic cell circadian', 'endothelial cell adhesion', 'tumor infiltration', 'CD8 T-cell clock', and 'circadian antitumor immunity', in combination with words such as 'clinical trials' and 'experimental data'. We also conducted a search targeting 'checkpoint inhibition time of day', 'vaccination time of day', and 'hematopoietic stem cell transplantation timing of day'.

Study selection and data extraction

All studies describing the time of CAR T-cell administration along with outcomes data (overall survival, progression-free survival, relapse, cell expansion/kinetics, and treatment-related toxicity such as ICANS) were included as clinical data evidence. We conducted a similar systematic review for experimental data providing direct evidence for a link between time of day and adoptive T-cell immunotherapy or providing an understanding of circadian regulation of immunity and T-cell responses (lymphocyte cell trafficking, dendritic cell activation/trafficking, adhesion events between T cells and other cells in proximity, tumor cell infiltration, CD8 T-cell clock mechanism). Review articles were primarily used for the purpose of secondary bibliography searching; they were not used as a source in cases where an original clinical/experimental article with sufficient detail existed.

For each of the three identified human studies of CAR T-cell treatment timing, we extracted the following: number of patients included, number of study centers, description of patient and disease population studied, type of CAR T-cell used in the treatment, description and distribution of the timing definition of infusion (e.g., morning vs. afternoon, actual time of infusion), all statistical adjustments carried out for treatment, and reported data for overall

survival, progression-free survival, tumor response/relapse rates, and cellular kinetic parameters. Toxicity data (i.e., incidence of grade >=3 ICANS, other adverse events that the authors report to potentially correlate with timing effects) were also meticulously collected.

Since we conducted independent reviews of three human studies reported on CAR T-cell infusion timing, and these studies, in addition to the differences in disease population and the CAR T-cell types/platforms used, also have heterogeneous characteristics in terms of duration definition of exposure, distribution and relative incidence of treatments in time windows, definition of outcomes, and statistical methods, we believe there is no statistically sufficient overlap among those cohorts to warrant any pooled statistical meta-analysis. We reported no pooled estimates from the analyzed papers.

Interpretation of evidence

The three clinical cohort studies were considered purely observational evidence in which there is an apparent correlation but not necessarily definitive proof of a causality-driven relationship based on circadian biology. The result of a null association study was never considered sufficient proof that time is totally irrelevant. The difference between a given time of administration in civil clock time and an actual chronobiological 'time of day' within an individual was thoroughly and directly examined because it inherently has significant implications for the accuracy of interpretation of 'timed therapies' as related to the biological phenomena driving treatment responses or toxicity profiles. The present article, aiming to propose a testable 'phase-alignment' hypothesis on CAR T-cell therapies, is based on a reinterpretation of publicly available results and was not prospectively tested in a clinical setting.

Outcomes

Three prospective multicenter trials provide direct evidence of human effects. Cumulatively, they consist of 2,351 subjects from 15 sites [18, 19, 20]. Two studies found later infusion to be associated with worse outcomes (these both assessed entirely different endpoints). The Lyons et al. trial of late infusion and survival is clear, while the two other trials examined the same progression or relapse endpoint at different times and came to contradictory conclusions, that is, that late infusion had a different impact on one of them.

Luan et al. looked at day-7 expansion, another indicator of response. No significant interaction was found at all for the Dowling et al. trial. Details of all three studies are presented in Table 1.

Study	Cohort and exposure	Main clinical result	Biological or toxicity result	Important caveat
Lyons et al. [18]	715 patients; 2 U.S. centers; time modeled continuously, including hours after local sunrise	Each hour later after sunrise was associated with higher adjusted odds of 90-day mortality; later time was also associated	Later time associated with grade 3-4 ICANS, anakinra use, and mechanical ventilation; no association with severe CRS	Retrospective. Only 36/715 infusions occurred after 15:00 in the categorical analysis; residual workflow or acuity confounding remains possible

		with shorter 2-year OS and EFS RMST		
Luan et al. [19]	1,052 adults with LBCL; 7 international centers; continuous time and before/after-noon analyses	HR 1.11 per hour for progression, relapse, or death; 1-y PFS 51.4% before noon vs 35.2% at/after noon; OS similar	Later infusion associated with lower day-7 expansion and higher peak inflammatory markers; no major toxicity difference	Retrospective. Baseline LDH and bridging therapy differed between timing groups; no direct circadian-phase measurement
Dowling et al. [20]	584 patients with LBCL; 6 Australian centers; multivariable analysis of infusion time	No independent association between infusion time and outcome after adjustment	No reported evidence supporting a clinically important clock-time effect	Retrospective. A null result may reflect a smaller true effect, restricted timing range, or absence of an effect; detailed biological phase was not measured

Table 1. Direct clinical studies of CAR T-cell infusion timing. Effect estimates are shown as reported in the source studies. OS, overall survival; EFS, event-free survival; RMST, restricted mean survival time; PFS, progression-free survival; CRS, cytokine release syndrome.

Lyons et al. looked at 715 adults treated at Washington University in St. Louis, U.S.A. (384 patients), and Oregon Health and Science University in Portland, U.S.A. (331 patients), from Jan. 2018 through Mar. 2025. The primary outcome of this trial was 90-day overall survival, although there were analyses at other intervals.

Infusion time was modeled continuously and with a time-to-sunrise function as used for many circadian biology studies.

The aim of using this metric, which accounted for latitude, season, and daylight-saving time, was to more accurately identify the relevant time of day.

Each hour later after sunrise was found to increase the adjusted odds ratio for the patient dying within 90 days by 24% [18]. For 365-day and day-90 survival, increases in the adjusted odds ratio per hour after sunrise were 15% and 24%. The adjusted odds ratio for 90-day mortality associated with each hour post-sunrise was 24% (95% CI, 1.04-1.47); the 365-day mortality HR was 17% (95% CI, 1.03-1.34); the HR for any grade III-IV ICANS was 24% (95% CI, 1.09-1.51); grade III-IV CRS was unaffected by infusion time.

For day 1-to-90 RMST, an extra hour later after sunrise was associated with 18 fewer days of survival and event-free status, respectively (2-year and event-free, -1.94; 2-year and event-free [95% CI, -32 to -6.1; -34 to -4], correspondingly).

Analyses performed by the investigators included extensive control for confounders: patient, disease, and treatment parameters and, where applicable, site as a random variable. This provided a respectable E-value between 1.35-1.57 with 95% CIs, and leave-one-hour-out analysis indicated that no specific one-hour interval uniquely accounted for an association with survival; the analysis did not highlight a problem. Inspection of 69 deaths between 0 and 90 days showed these to have occurred secondary to malignancy and sequelae from treatment, irrespective of time to treatment infusion. Post-thaw hold time revealed no correlation between it

and the time of infusion; in the instances in which clinical records of late >15:00 infusions were available, there was no reason to account for both deviation to infusion time and inferior outcome [18].

It must be stated that all interventions in this trial were retrospective, and whilst the statistical controls were stringent, this does not account for residual confounders, such as an underlying condition dictating the timing of infusion and influencing prognosis.

Luan et al.: measured survival, PFS, and day-7 CAR T-cell expansion. There were 715 adults with relapsed or refractory LBCL who received CD19 CAR T-cell infusions within the framework of this multinational multicenter study. 1,052 subjects are included, with treatments from Jan. 2017 until April 2025 across seven centers worldwide. Subjects were all adults receiving therapy for relapsed/refractory LBCL. Mean infusion time was 11:48 a.m. (median; IQR, 11:06 a.m.-12:45 p.m.; mean range, 09:00 a.m.-6:45 p.m.).

The demographic and baseline clinical features were similar between early and late infusion-time groups, though in the late group elevated LDH, in combination with reduced bridging therapy, was seen more frequently.

Controlling for key differences in patient-, disease-, and study-related variables in multivariable models [19], the death HR for each hour later was 1.11 (1.03-1.20; P = 0.004) for progression or relapse (the event of interest was any progressive or relapsing disease) [19]. One-year overall PFS rate was higher in those treated earlier compared to later, at 51.4% and 35.2%. Survival data were also presented for 2-year median 2Y RMST (survival; 95% CI), which is higher with earlier infusion time. A more detailed analysis looking at day-7 response showed better 1-day response (CAR T-cell expansion on day 7 is higher when injected in the morning, P = 0.04).

CAR T-cell expansion at day 7 did, however, seem to be influenced by the time of day. An increase was observed in day-7 CAR T-cell numbers (day-7 CAR T-cell expansion in day 1 [higher in morning dose], P = 0.002) [19]. No differences were identified for major immune toxicities between the groups.

These day-7 cellular data do offer an insight into whether the time of day affects overall CAR T-cell growth, but this does not prove causality in terms of their contributing to the differences seen in PFS.

Furthermore, for these metrics to be therapeutically meaningful, a greater understanding of post-thaw viability and handling in general is necessary. Again, a simplistic division of midday versus the remainder of the day must be regarded with caution. There are some issues in the data on time of day, for example, if local time of infusion implies a range only between 11 a.m. and 1 p.m. Dowling et al.: analyzed multiple outcomes, none linked to time of infusion. Dowling et al. carried out a trial looking at the impacts on response, remission rate, relapse, death, and 90-day MCITS within six clinical centers in Australia. There were 584 participants. After controlling for many measured confounding factors, the study found no independent association between CAR T infusion time and clinical outcome [20].

This finding that U.S. or European results could not be replicated within an Australian cohort is therefore a significant observation.

This finding is, however, unable to differentiate a true lack of effect in relation to timing from an effect that may exist at smaller values or be affected by patient group or product. It also cannot completely negate the effect of residual confounders despite stringent multiple adjustments.

Circadian mechanisms are likely important in the first hours of therapy.

Research has identified numerous pathways by which daytime affects T-cell activation: the circadian clocks resident within lymphocyte populations regulate entry into immune tissues and influence their adaptive responses [8]. Dendritic-cell migration through lymphatic vessels is subject to diurnal change, as is antigen presentation, resulting in differential activation potential of the subsequent T-cell response [10]. The tumor sites themselves are also subject to changes according to the circadian clock, including immune-cell infiltration [11-12] and dendritic-cell activity [11]. Host circadian rhythm deregulation negates these changes in tumor biology.
All the above could be applicable to clinical trials, insofar as CAR T cells must transit from the vascular space to the disease site in order to engage with antigen.

Data to support actual cellular events impacting the clinical studies remain largely unavailable, with the day-7 cell count merely being indicative of a process that, whilst important, accounts for a complex combination of factors including cell count at injection, disease level, inflammation, and activation [5-7].

What remains measured is unclear.

None of the conducted trials analyzed the effect of melatonin phase, cortisol phase, phase as related to resting-behavior ratio, or other markers that objectively would inform an individual's intrinsic phase. The sunrise function used in the work of Lyons is certainly a better measure than civil time, yet fails to portray an individual's accurate position within the cycle [18]. Sleep cycles, acute illness (fever), as well as steroid treatment and time within the hospital, affect time of day of infusion.

Post-thaw handling of CAR T cells was specifically investigated by Lyons in their cohort and demonstrated nothing contrary to this day-specific effect, but no study measured the effect of cell state against phase on the receiving patient.

Discussion

To date, no generalizable rules support daytime administration over later periods for CAR T-cell infusion. In two multicenter studies, positive associations with later timing of infusion were clinically significant, and a third multicenter study revealed no difference [18-20]. Furthermore, the two positive studies showed divergent changes in that the Lyons study reported clinical outcomes, namely progression and a select number of adverse-event signaling outcomes [18], while the Luan et al. study did show improved progression-free survival in conjunction with reduced day-7 expansion [19].

However, future research should consider this heterogeneity as opposed to averaging out data.

Clock time and circadian phase are not biologically the same thing.

One plausible cause for heterogeneity in the data is the definition of exposure measurements taken. While the electronic record contains civil time and allows for easy tracking of this civil time for every patient, the same cannot be said for circadian phase. Individuals administered a treatment within the same hour of the day can have wildly different sleep habits, melatonin phases, and hence chronotypes, as the phases will also shift depending on the time of year [25]. In addition, numerous patient-care-related activities, particularly at night with constant bright lighting, serial awakenings for vitals check-ins and scheduled medications (particularly those involving corticosteroids), fevers, and irregular food intake at inappropriate hours may shift and hence alter inherent patient circadian rhythms.

This sunrise-related time in the Lyons study aligns with an exposure measurement artifact [18], without necessarily proving a circadian rhythm causal link. Indeed, as noted above, the sunrise itself only serves as a single time indicator of an internally functioning oscillator. Rather than comparing time of day between civil hour markers, we would need a prospective RCT tracking both civil time and biological time (e.g., melatonin phase), prior to hospital admission, following it, and then through the hospitalization and during the treatment period itself. The remaining significance after replacing civil clock time with biologic clock time in this comparison would serve to strengthen that supposition.

In the context of this putative study, we employ a second name that circumvents issues when discussing comparing times across a civil clock and/or between a given civil clock and circadian phase, i.e., we introduce what we have herein named 'phase alignment,' in which, during each infusion, it is a temporal nexus involving the CAR T-cell product, the treated patient, both vascular and tumoral, where both timing relative to both and relative time to each other, not only of the patient but also of the specific product delivered (as has been posited in this and other papers of ours), are brought to bear; this is not necessarily with explicit assumptions regarding precise timings occurring at that time (as, after all, identical civil clock hours may correspond to different biological time periods both relative to the characteristics of the receiving treatment center and the patients themselves).

Day-7 expansion is arguably a more temporally relevant marker of outcome:

We demonstrated herein that day-7 expansion data from the Luan cohort may serve as a relevant tool to evaluate hypothesized timing of CAR T-cell administration [19]. For if timing dictates infiltration and early cellular activation, the blood concentrations of CAR T cells can be expected to be different prior to their definitive therapeutic effect on progression-free survival. This hypothesized sequential relationship is testable with early CAR T-cell blood draws immediately prior to infusion and periodically for the initial week to two weeks of treatment, allowing us to define the start of difference at various time points by tracking early CAR T-cell amplification/attenuation at respective time points and the effect of such events on progression-free survival (mediation analyses).

If no association is seen between an individual's circadian phase and a correlation between CAR T-cell phase and progression-free survival, it may reflect other biological factors that affect outcome, such as host inflammation (the primary, circadian-driven component, or it might act in concert with the infused product), or time associations depending on concomitant chemotherapy or on a particular molecular target of the CAR T cells. Information on differences in treatment protocols between comparative groups has not been readily available to compensate for such potential confounding issues in the reported associations.

CAR T-cell product and disease compartment can modulate the effect of timing interactions.

CAR T-cell products are characterized by heterogeneity of their expansion kinetics: with CD28 costimulation, the CAR T cells expand much more quickly and the CAR T cells are shorter-lived, whereas costimulation through 4-1BB results in a lower and delayed expansion phase that is followed by more prolonged function in the patient [6, 7]. If the duration after infusion of the product is indeed meaningful early on with activation in vivo, the differential expansional differences between different cell products need to be taken into account. This requires examining potential interaction effects such as the time course at which particular types of CAR T-cell products cause amplified effects on their disease environment, rather than adjustment for product in regression equations, which can obscure nonlinear time-dependent interactions. Disease compartment is another such aspect: infiltrating the solid tumor found in Hodgkin's lymphoma is a distinctly different clinical event than targeting leukemia cells present in the periphery or infiltrating the bone marrow specialized in myeloma therapies. Such mechanisms are distinctly different and may or may not be associated with timing differences dependent on the specific disease location; the current study may have combined such varying disease types and thus masked subtle findings that could have emerged only by looking at such data in an additive way over groups separated by cell source and disease process.

Timing may affect both efficacy and toxicity differently.

Lyons et al. reported increased severe ICANS accompanied by mechanical ventilation but not CRS toxicity in the same population treated with later-occurring timed infusion of CAR T cells [18]. This difference should not imply distinctly separate circadian-triggered events to explain an increase in toxicity. However, it does dispute a simplistic circadian control for a generally heightened systemic inflammatory response at varying timed exposures. Neurotoxicity, unlike CRS, is linked to increased inflammatory processes directed at cerebral blood vessels and BBB disruption. Cellular events may be at play with each form of toxicity that affect biological circadian rhythms. These different events should lead to separate timing assessments for efficacy vs. toxicity, and for this we have designated the terms efficacy window and neurovascular vulnerability window. The efficacy window will measure if early trafficking of CAR T cells is associated with specific timing patterns or expansion trajectories, and later efficacy endpoints such as clinical response and progression-free survival will then correlate time to these early parameters, whereas the neurovascular vulnerability window will examine correlation with later events that lead to neurologically damaging toxicity such as ICANS. It is hypothesized that such

time-response curves may differ or that one of them will likely be nonexistent in humans. Figure 1 summarizes this proposed phase-alignment model and illustrates the potentially distinct timing relationships between CAR T-cell efficacy and neurovascular toxicity.

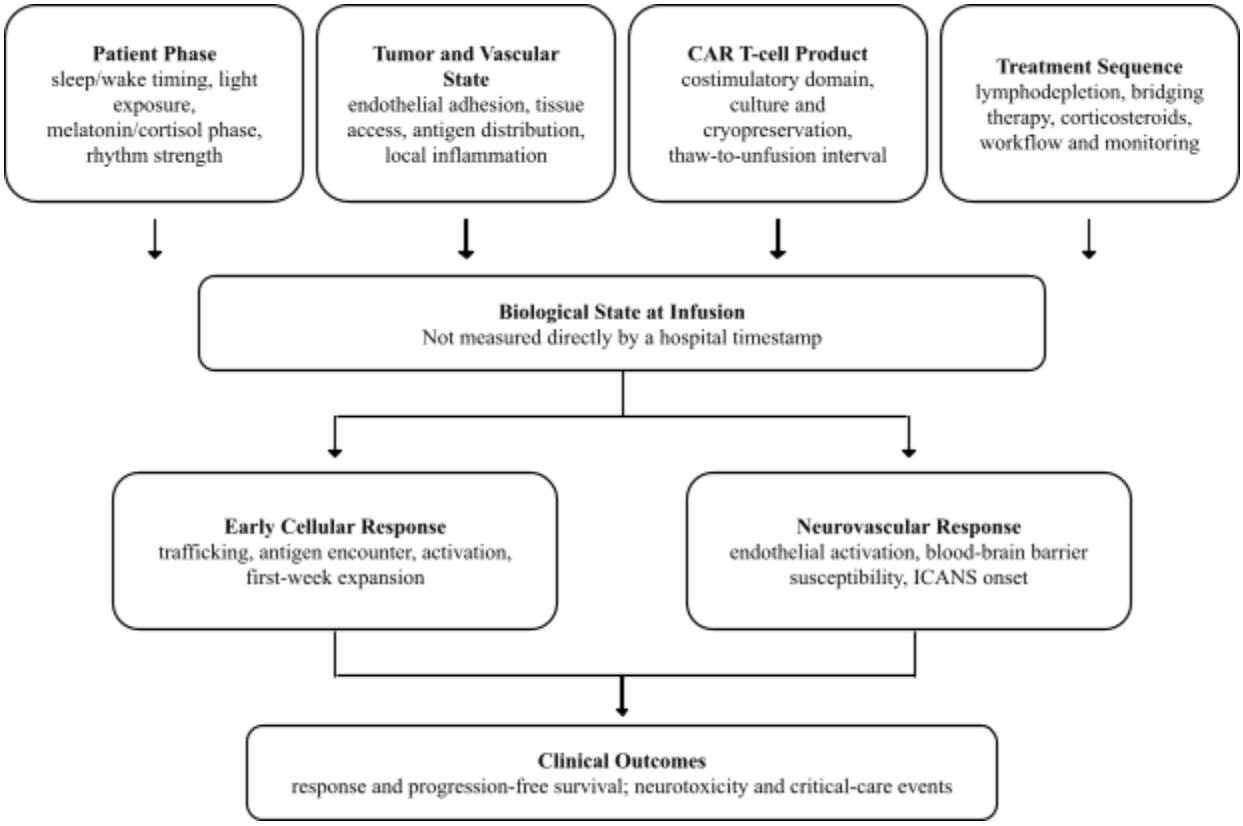


Figure 1. Working model for phase alignment at CAR T-cell infusion. The hospital timestamp captures only one part of the state at infusion. Patient circadian phase, the tumor-vascular environment, the cell product, and the preceding treatment sequence may contribute separately or interact. The proposed early cellular and neurovascular responses are hypotheses to be tested, not established pathways in patients.

Clinical workflow can lead to the same statistical pattern.

The question may also be framed around circadian biology. However, the three clinical studies did not explicitly test for it. Instead, some authors may have sought other justifications (even if less than perfectly rigorous) for later infusions, e.g., as a proxy for when a particular product became available for release testing; to complete other pre-infusion evaluation steps; because pharmacy access, bed availability, or staffing caused delays; or as a marker for higher acuity and quality of postinfusion care.

These are common workflow issues, and each could be linked to either the prognosis or the degree of effect.

Adjustment can diminish, but not erase, the bias created by unrecorded differences between late and early infusions or by workflow related to patients with a poor prognosis. Fortunately, a more comprehensive job of controlling for this, particularly with day-of-infusion

severity and setting and subsequent investigation of late infusions, was undertaken in one of the most published series of studies [18]. Those studies are therefore less parsimonious a source for simple scheduling changes, but they still do not convert analyses based on real-world workflow into evidence from randomized trials. The existence of one of the neutral studies of infusion order does provide a vivid example of how differing center characteristics and workflows might alter, even nullify, a biological finding or a potential bias [20].

Design of a prospective trial

I propose a prospective randomized trial where possible, perhaps in a multicenter setting. Randomization within a narrow, clinically acceptable range to an earlier versus a later treatment time is the ideal trial.

This can be random, to the hour, for the treatment timing. With consideration of both product type and center, some randomization will also occur based on day of the week; thus, a balance will not be ensured over 1 to 2 weeks. However, such randomness provides a test of whether the scheduling itself has an effect (and whether it might be important to optimize clock time).

Additional non-randomization would concern whether and when there is a measured circadian rhythm. If these events are recorded at the same time within the patients as during the infusion, it will help differentiate a scheduled time effect versus a measured time phase. Some patients might need to wait the full scheduled window (as would typically occur within 3-7 d depending on the cycle), as all patients need the time slot allocated.

Ideally, the specific product is part of the block design in a study that randomizes timing; perhaps the choice is a balance of a product's typical infusions or of whether time of day has a particular clinical trial associated.

A more sophisticated design would also involve a non-random sample or randomly timed patients whose circadian time is measured in more detail, or measuring circadian time to see if it would have better predictive value for outcomes compared to actual infusions given by clock time. These may all occur in the same patient if timing to within an hour will be used.

Circadian measurement

A comprehensive circadian measurement, including at least sleep and light and even physiological measurements like those from the use of an actigraph on a wristwatch (although it may not work well with actively ill patients), is the way to capture circadian measurements at the time of measurement. Circadian biology is not the only rationale for positive findings; however, other circumstances might lead to later administration, such as when delivery, release testing, or pharmacy workup may be delayed to the next delivery cycle due to product backlogs.
Another major reason for delay often cited by investigators in clinic reports in the negative study series is that patients need more complete workups.

Thus, the correlation might just occur if disease severity is related to the quality of follow-up care and, of course, the quality of treatment. Any subsequent analyses must be

consistent for the sake of better comparisons between early and late doses, as this will also help eliminate the bias of many factors.

Measuring circadian biology

The challenge remains that until this work, no one has collected circadian time, at least on the day the patients may need the infusion; yet product-line-specific delays for clinical reasons do account for some of the study data.

Nonetheless, more and more medical specialists are taking the circadian rhythm and its biology into consideration. Measuring circadian and biologic states in patients undergoing these treatments is necessary in all cases. Early infusion times will have patients coming into hospitals and having treatments at many points, even if within an assigned or agreed-upon time, given the patient's needs or other demands for beds or services.

Thus, a trial to explore this area should be more sophisticated than the initial work presented so far and include, among other things, measurements of patients using a standard rhythm measurement.

Treatment and workflow variables

A thorough analysis of clinical workflow variables, the nature of which has also been previously mentioned, should include: the time the product became ready to ship; the time the product was shipped; the time the product arrived at the infusion facility.

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Gut-Brain Axis: Understanding the Microbiome Dysbiosis in Alzheimer's Disease Pathogenesis, Progression, and Therapeutic Interventions By Alya Ural

Abstract

Dementia is an increasing global health crisis with an estimated 57.4 million individuals living with the condition in 2019, and projected to rise to 152.8 million in 2050. Yet, current medical interventions offer limited disease-modifying benefits, prompting further empirical inquiries into the peripheral contributors to neurodegeneration. Evidence so far implicates gut microbiome and gut–brain axis in Alzheimer disease pathogenesis. Age-related dysbiosis also correlates with inflammation, dysfunction of the blood brain barrier, microglial activation, and accelerated amyloid-β deposition. Possible interventions that have been proposed include probiotics, prebiotics, and Mediterranean-based diets, as well as experimental fecal microbiota transplantation which have been reported to restore microbial balance in animal models.

1. Introduction

Dementia is an increasing global challenge, noted as a significant cause of disability in individuals over the age of 65. The number of cases is also expected to grow, with a projected increase of cases from 57.4 million in 2019 to 152.8 million in 2050. It was originally ranked the ninth cause of national death in 1990, and then rose to become the 7th cause in 2017, and is predicted to become the second cause by 2040. Dementia is a syndrome with a large range of symptoms, including memory and language impairments, as well as significant cognitive decline (Arvanitakis et al.). The most common root of dementia is known as Alzheimer's disease (AD). AD is a neurodegenerative disorder that has very few clinically successful disease-modifying therapies (Chandra et al.), leaving a large number of patients untreated when it comes to stopping or slowing down the effects.

In addition to focusing on the central nervous system, recent research has started on identifying the impacts of the gut-brain axis on cognitive function (T. Zhang et al.). In the GI tract, there are numerous different organisms, including bacteria, eukarya, archaea, that compose the gut microbiome. The development of the gut microbiome takes place starting at infancy. After birth, the GI tract becomes colonized with illness, diet, and antibiotic treatments often changing the microbiota. The composition of the microbiota is dependent upon environmental factors as well as the host, and oftentimes the longevity of microbes is reliant upon its usefulness in improving the host’s fitness. These microbes mainly help to keep the gut-lining healthy, protect against pathogens, and to provide nutrients. Additionally, the communication between the microbiota and the mucosal immune system is crucial to maintain overall proper immune function (Thursby and Juge). Recent evidence suggests that the gut microbiome (GMB) may have a rising role in the neuroinflammation and acceleration of amyloid deposition, thereby inducing the progression of AD in human patients (Chandra et al.). By integrating current human and animal clinical evidence, this review paper aims to deliver an in-depth understanding of the gut-brain developments. It will highlight how microbe-targeted treatments could help to transform the medical approach to combatting the progressing neurodegenerative crisis.

1.1 Alzheimer's Disease: History and definition

Alzheimer's is one of the most common forms of dementia, ranging from severities of cognitive impairment through incompetence. This neurodegenerative disease impacted over 6 million individuals above age 65 in the United States in 2020 and this number is expected to increase to 13.85 million Americans by 2060 (T. Zhang et al.). Alzheimer's disease was first presented by the German clinical psychiatrist and neuroanatomist Alois Alzheimer in November 1906. Starting in 1901, Alzheimer began to document and investigate the case of Auguste D. at the Frankfurt Psychiatric Hospital (Hippius and Neundörfer). After the passing of Auguste, Alzheimer began to analyse the patient's brain. He soon identified a profound loss of neurons as well as numerous physical abnormalities that he then presented to the medical community in 1906 (Shim et al.). Despite the initial identification of these physical abnormalities occurring in 1906, the precise molecular identities remained unknown until 1984. In 1984, researchers successfully characterized the amyloid beta (AB) peptide that combines to form extracellular plaque (Glenner and Wong). Shortly after 1986, scientists discovered that the atypical hyperphosphorylation of the protein tau was the fundamental component of the neurofibrillary tangles associated with the disease (Grundke-Iqbal et al.).

1.2 Symptoms

The clinical progression of AD is known as the gradual decline of the episodic memory (Dubois et al.). In the earliest presymptomic stage, the disease begins from small memory lapses as well as subtle pathological alterations in the hippocampus and cortex (Shim et al.). During these early stages, patients typically maintain normal daily functioning without exhibiting any clinically apparent cognitive deficits (Sperling et al.). As the disease approaches the early symptomatic phase, individuals begin to experience difficulties with memory, focus, and certain executive functioning tasks (Tarawneh and Holtzman). Progressing into the moderately symptomatic period of AD, patients will experience deeper neurodegeneration, causing physical impairments and severe cognitive declines. This includes being unable to recognize family, having communication barriers, a loss of impulse control, and having hallucinations (Breijyeh and Karaman). By the final, most severe stages of AD, neuropathology spreads across the full cerebral cortex with massive accumulations of amyloid-beta (AB) and tau proteins. This leaves patients bedridden, unable to swallow, speak, or manage basic bodily functions (Breijyeh and Karaman). In addition to the classic memory-centric symptoms, early onset patients may experience abnormal variants including logopenic progressive aphasia (LPA), posterior cortical atrophy (PCA), and a frontal variant AD (Polsinelli and Apostolova).

Patients diagnosed with PCA often face dyspraxia and severe visuospatial challenges, while their memory is still spared during the earlier stages(Polsinelli and Apostolova). Conversely, patients who showcase LPA or a frontal variant of AD often experience extreme language barriers, rapid cognitive decline, and behavioral changes (Polsinelli and Apostolova).

1.3 Pathology: Genetics factors and Mechanism

At the structural level, AD includes two types of cerebral changes: positive and negative lesions (Serrano-Pozo et al.). Positive lesions refer to the atypical accumulation of protein aggregates like amyloid-beta (AB) proteins and neurofibrillary tangles (NFTs). Meanwhile, negative lesions represent structural loss driven by neuronal and synaptic degradation (Serrano-Pozo et al.).

The main neuropathological hallmarks that define the disease are the extracellular aggregation of AB peptides into plaques as well as the accumulation of hyperphosphorylated tau protein (Serrano-Pozo et al.). The AB deposits come from the cleavage of the amyloid precursor protein (APP), which is a type 1 transmembrane glycoprotein (Pfundstein et al.). APP proceeds through the amyloidogenic pathway, which is cleaved by the enzymes y-secretase and b-secretase to provide AB monomers (Hur). The homeostatic disturbance of this peptide causes it to become soluble oligomers, which may subsequently become insoluble amyloid fibrils and senile plaques (Hampel et al.). An accumulation of these aggregates can cause extreme synaptic dysfunction and inflammatory responses that damage surrounding neurons (Fanlo-Ucar et al.). Alongside the plaque formation occurs the development of NFT's, which are composed of aggregates and irregularly hyperphosphorylated tau proteins (Boutajangout et al.). Tau is encoded by the MAPT gene on chromosome 17 and works to combine and stabilize the microtubule network that is imperative for internal cellular transport (Kovacs).

When tau becomes overly phosphorylated, it can lose its normal ability to bind to microtubules, which can contribute to microtubule instability and disrupted intracellular transport (Kent et al.). The detached tau proteins can then turn into insoluble structures that contribute to the creation of neurofibrillary tangles within neurons (Yang et al.). These tau-related changes can damage axonal transport, interfere with neuron structure, and contribute to neuronal death and dysfunction (Zubčić et al.). Neurofibrillary tangles and Amyloid plaques are found throughout the brain, mainly in the hippocampus and the parts of the cerebral cortex involved in cognition and memory (Furcila et al.). As these abnormalities spread, they are followed by neuronal degeneration, progressive cerebral atrophy, and synaptic loss (Henstridge et al.). Regardless of the extensive research, scientists have not determined the exact order of the biological events at fault for the development and progression of AD (Reitz). One widely circulated explanation is the amyloid cascade thesis, although researchers continue to debate whether or not it can fully explain the complexity of the disease (Reitz). The hypothesis states that the imbalance between the production and removal of AB causes it to accumulate in the brain and therefore initiate more pathological changes (Tarasoff-Conway et al.). According to the hypothesis, AB aggregation can cause inflammation, oxidative damage, synaptic dysfunction, altered tau activity, and neuronal death (X. Sun et al.). The relationship between cognitive impairments and amyloid deposits isn't always direct, showcasing that several biological mechanisms likely contribute to AD (Nelson et al.). Therefore, although AB plaques and neurofibrillary tangles remain the defining pathological features of AD, the ways which these irregularities interact to drive disease progression are still not entirely understood (Paula et al.). Current medications can manage certain symptoms or slow disease progression in certain

patients, but there is no available complete cure for AD or any intervention that can reverse the neuronal damage it causes (Wang et al.). New methods such as optogenetics may help researchers study damaged neural circuits and investigate ways to restore cognition in certain models of AD (Mirzayi et al.).

2. Gut Microbiome and the Brain Axis

2.1 Microbiome Development

The gut microbiome is a complex biological community that contains trillions of microorganisms that begin to develop early on in life and continue to alter throughout the course of life (Dziedziak et al.). Numerous factors impact the contents of the gut microbiome such as lifestyle, medical conditions/medication use, the method of birth, and diet (Dong et al.; Dziedziak et al.). In healthy adults, the microbiome is mainly composed of Firmicutes and Bacteroidetes, with a bit of Verrucomicrobia, Proteobacteria, Actinobacteria, and others

Aging can significantly impact the microbial contents (Z.-J. Zhang et al.). Adults above the age of 65 often have decreased levels of beneficial bacteria, like Bifidobacterium, as well as reduced overall microbial diversity (Z.-J. Zhang et al.). The aging microbiome may be less productive at creating short chain fatty acids and fermenting nutrients because of less digestive function, certain medication use, a reduced variety in diet, lowered physical activity, and physiological frailty (Z.-J. Zhang et al.). Combined, these changes can increase the chances of dysbiosis, which is an imbalance in the composure and function of the gut microbiome (Z.-J. Zhang et al.).

Dysbiosis has been correlated with AD and cognitive decline (Dong et al.). Studies that compare individuals with AD to individuals who are cognitively healthy have found less microbial diversity as well as differences in the presence of bacteria among those who have the disease (Vogt et al.). These microbial changes have been associated with Alzheimer's biomarkers, showcasing that age-related alterations of the gut microbiome may lead to the neurological changes demonstrated in disease progression (Ferreiro et al.). This connection provides a mechanism in which intestinal changes are able to impact the growth of cognitive symptoms (Dong et al.).

2.2 Gut-Brain Communication Pathways

The gut-brain axis is a bi-directional communication system that links the gastrointestinal tract and the central nervous system (M. Sun et al.). This communication process occurs through a network of mechanisms including immune, neural, metabolic, and endocrine signaling (M. Sun et al.).

2.3. Neural Pathway

The neural pathway connects the gut and brain via the enteric nervous system and its connection to the central nervous system (M. Sun et al.). The vagus nerve is an important route

within the system because it carries signals between the gastrointestinal tract and the brain (M. Sun et al.). Through this path, intestinal activity can influence brain processes related to behavior, cognition, memory, and emotion (M. Sun et al.).

Research also shows that gut microorganisms can impact brain development and function (Lin et al.). Germ-free mice have shown differences in microglial development, hippocampal neurogenesis, as well as amygdala structure (Lin et al.). Since the amygdala and hippocampus support memory and emotion, these findings support a potential correlation between microbial signs and neurological activity (Lin et al.).

2.4. Immune Pathway

The immune pathway is also important because a primary function of the gut microbiome is to monitor immune activity and maintain the intestinal barrier (Dong et al.; Lin et al.). When dysbiosis weakens the barrier, microbial products create systemic inflammation (Lin et al.). These inflammatory signals can affect the central nervous system and contribute to neuroinflammation, which is a key element of Alzheimer's disease (Lin et al.).

Gut microorganisms also influence microglia, which are the immune cells of the central nervous system (Lin et al.). In germ-free mice, microglia showcased weaker responses to infection, displaying that microbial signs are essential for normal immune function (Lin et al.). Therefore, dysbiosis may contribute to Alzheimer's related inflammation by disrupting the intestinal barrier and by impacting both microglial and immune activity (Dong et al.; Lin et al.).

2.5. Metabolic Pathway

The metabolic pathway involves outputs created by the gut microorganisms that can alter barrier integrity, immune regulation, and brain function (Dong et al.). Short-chain fatty acids (SCFAs) are created when bacteria ferment dietary fiber and other carbohydrates (Dong et al.). Major SCFAs include propionate, butyrate, and acetate (Dong et al.).

SCFAs also have the ability to change the blood-brain barrier and microglial function (Dong et al.; Lin et al.). Research in germ-free mice has shown that the lack of gut microorganisms can increase the permeability of this barrier, while microbial colonization or butyrate treatments can lessen this effect (Lin et al.). Aging-related declines in beneficial bacteria may decrease SCFA production (Dong et al.).

On the other hand, lipopolysaccharides, or LPS, are inflammatory components that are associated with Gram-negative bacteria (M. Sun et al.). If dysbiosis increases barrier permeability, greater exposure to LPS may cause neuroinflammation (M. Sun et al.). Together, reduced SCFAs and increased exposure to inflammatory microbial products may create conditions that support the progression of AD (Marizzoni et al.).

3. Mechanisms Linking Gut Microbiome to Alzheimer’s Disease

The gut microbiome can contribute to AD through several different mechanisms (Chandra et al.; Seo and Holtzman, “Current Understanding of the Alzheimer’s

Disease-Associated Microbiome and Therapeutic Strategies"). Dysbiosis can change systemic inflammation, the strength of the blood-brain barrier, microglial activity, the production of metabolites, and protein aggregation (Chandra et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies")). However, much of the current evidence regarding the mechanistic aspect of AD originates from animal and cellular studies, meaning that researchers are still determining how impactful these mechanisms are to humans(Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies").

3.1.1 Neuroinflammation

One of the primary ways microbial dysbiosis connects to AD is through neuroinflammation (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). A strong immune system and healthy intestinal barrier are dependent upon a balanced microbiome (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Dysbiosis causes the intestinal barrier to become more permeable, which allows certain inflammatory signals and microbial metabolites to enter the bloodstream (Sochocka et al.). These signals can be elements of outer membrane bacteria that might trigger inflammatory immune responses, including LPS (Sochocka et al.). Increased systemic inflammation can change the central nervous system via immune-cell signaling, alterations of the blood-brain barrier, and cytokine circulation(Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies").

External inflammatory molecules do not need to enter brain tissue to have an effect on the brain (Dong et al.). They can communicate with endothelial cells at the blood-brain barrier or trigger signaling paths that can change the actions of the astrocytes and microglia (Dong et al.). Microglia are the brain's immune cells and they help to respond to infections, remove cellular debris, and assist with tissue maintenance (Dong et al.). However, a constant inflammatory state can shift microglia to be in a chronically activated state, thereby releasing inflammatory cytokines and reactive oxygen species (ROS). This relationship can overall intensify neuronal stress and sustain a cycle of inflammation (Dong et al.).

This inflammatory cycle is relevant to both pathological features of AD. When glial cells get stuck in an overactive state, they stop clearing out amyloid-beta effectively and release substances that raise the levels of amyloid toxicity (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Constant inflammation can support tau hyperphosphorylation and the development of tau-related neurodegeneration (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Animal studies have showcased changes in microglial gene expression and morphology tied to gut microbiome composition (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). These experiments showcased reductions in amyloid deposition and tau

pathology, thus leading to decreased levels of neurodegeneration (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Regardless, these results should not be understood as proof that dysbiosis can be the root of AD in humans, rather it should be understood that dysbiosis can be a factor within disease progression (Chandra et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies").

3.1.2 Amyloid and Protein Misfolding Pathways

The gut microbiome also has the ability to impact AD through amyloid and protein-misfolding pathways (Chandra et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Many bacterial species yield amyloid proteins that help them bind to surfaces, survive environmental stress, and form biofilms (Chandra et al.). One specific example of this is a curli, which is a bacterial amyloid fiber created to help with surface-binding and building biofilm structure (Chandra et al.). Even though bacteria amyloid are not identical to human amyloid-B, both can create B-sheet rich structures (Chandra et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Their structural sameness has led researchers to examine whether long-term exposure to microbial amyloids may cause the immune system to respond abnormally to misfolded proteins(Chandra et al.).

One suggested mechanism is molecular or immune priming (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Exposure to bacterial amyloid can trigger pattern-recognition receptors and boost inflammatory responses that later cause the aggregation or reduced removal of amyloid-B in the brain (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Microbial amyloids could, therefore, contribute through sustained inflammatory conditions favorable to protein misfolding(Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Another proposed mechanism is cross-seeding, where one misfolded protein promotes another protein to foster an abnormal aggregated structure (Friedland and Chapman). However, direct evidence that bacterial amyloids travel from the human intestine and initiate amyloid-B plaques in the brain remains scarce (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). The mechanism is therefore biologically reasonable but is not fully settled in human AD (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies").

Animal studies supply additional evidence that the intestinal microbiome can impact cerebral amyloid deposition (Chandra et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Germ-free AD model mice and some antibiotic-treated mice showed noticeably less amyloid buildup compared to traditionally raised controls (Chandra et al.). In some experiments, transferring microbiota from AD model animals into germ-free mice increased cerebral amyloid deposition (Chandra et al.).

These results propose that microbes have the ability to impact amyloid-related processes (Chandra et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). However, the results may vary depending on the animal model, showcasing how these findings cannot directly be applied to humans (Chandra et al.).

3.2 Microbial Metabolites and Neurotoxicity

Microbes produce metabolites that can enter the circulation and influence nervous-system, immune, and metabolic activity (Loh et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). These substances include SCFAs, tryptophan metabolites, trimethylamine N-oxide, bile-acid derivatives, and microbial inflammatory products (Loh et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Dysbiosis can shift both how much of these substances is produced and the ratio between them, which in turn may alter whether they help or hurt the host (Loh et al.).

3.2.1 Short-Chain Fatty Acids (SCFAs)

SCFAs are created mainly when intestinal bacteria ferment dietary fibers (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies") . The primary SCFAS include propionate, acetate, and butyrate (Abdel-Haq et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies") . Under healthy conditions, SCFAs present energy to intestinal cells, monitor immune responses, and assist in maintaining intestinal-barrier strength (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). They also engage in the progression and regulation of microglia, showing that microbial metabolism can influence immune activity within the brain (Abdel-Haq et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies").

However, the role of SCFAs in AD cannot be classified as "black or white", i.e., harmful or helpful because it is heavily dependent on context (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies") . Butyrate has showcased anti-inflammatory and barrier-supporting qualities in certain studies, and some butyrate treatments have been connected to less amyloid pathology (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies") . In contrast, combined SCFA addition in germ-free AD model mice accelerated microglial changes and increased amyloid-B plaque deposition (Colombo et al.). Research has showcased that the effects can change depending on numerous factors, including the specific type of the SCFA (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies").

SCFAs may affect microglia through epigenetic management and immune signaling (Colombo et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Acetate can enter the central nervous system and engage in microglial metabolic processes, while certain SCFAs can also monitor cytokine production through peripheral immune cells (Colombo et al.; Erny et al.). Thus, a changed SCFA composition could change the level of neuroinflammation and the capability of microglia to respond to amyloid-B (Colombo et al.). These results showcase why the balance of microbial metabolites is more important than framing the SCFAs as either protective or harmful as they are heavily context-dependent (Colombo et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies").

3.3 Blood–Brain Barrier (BBB) Dysfunction

The blood-brain barrier is created by certain endothelial cells, pericytes, tight junctions, perivascular feet, and other elements of the neurovascular unit (Knox et al.). It monitors the movement of substances between the blood stream and central nervous system, supporting the protection of neurons from toxins, pathogens, and extreme inflammatory exposure (Knox et al.). Aging can impair a number of components of this system, while neurodegenerative diseases can cause additional barrier and vascular irregularities (Knox et al.).

Gut dysbiosis can contribute to blood-brain barrier dysfunction by promoting intestinal permeability and systemic inflammation (Loh et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). LPS, circulating cytokines, and other microbial byproducts can impact tight-junction proteins and endothelial cells, possibly making the barrier more permeable (Loh et al.). An impaired barrier can allow for more communication between peripheral inflammation and the brain, higher immune-cell recruitment, and expose neural tissues to substances that are typically restricted (Loh et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies").

Blood-brain barrier damage can also impact amyloid-B clearance and cerebral blood flow (Knox et al.). The consequent environment can endorse glial activation, synaptic dysfunction, oxidative stress, and neuronal injury (Knox et al.). Therefore, barrier dysfunction can be both a consequence of aging and AD pathology and a mechanism that allows peripheral microbial disturbances to accelerate neurodegeneration (Knox et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies").

3.4 Microbiome–Brain Aging Interaction

Aging impacts both the brain and the gut microbiome, making sure that their paths intertwine with one another (Abdel-Haq et al.; Chandra et al.). Older adults may have constant inflammation, a worsened intestinal barrier, and less microbial diversity (Chandra et al., 2023). Concurrently, aging microglia can become more responsive and less productive at sustaining

neural homeostasis (Abdel-Haq et al.). An age-changed microbiome can thereby boost an already vulnerable inflammatory and metabolic environment (Abdel-Haq et al.; Chandra et al.).

Microglial dysfunction can also disrupt synaptic pruning, which is a process through which damaged or needless synaptic connections are removed (Zhao et al.). Even though pruning is needed for brain care, extreme microglia-driven pruning may promote cognitive impairment and synaptic loss (Zhao et al.). Recent research showcases that gut-derived bacterial extracellular vesicles transporting LPS can trigger microglial activation and inordinate synaptic pruning (Zhao et al.). This can provide a possible pathway through intestinal microbial products that have the ability to impact synaptic stability (Zhao et al.).

Lastly, dysbiosis has the ability to raise levels of oxidative stress and disrupt mitochondrial function (Zhu et al.). Microbial metabolites and inflammatory signals can affect mitochondrial energy production, cellular stress responses, and oxidative bursts (Zhu et al.). Mitochondrial dysfunction can reduce neuronal energy and increase oxidative damage (Zhu et al.). This relationship creates a feedback loop of which microbial imbalance feeds inflammation, which stresses mitochondria, which damages synapses, which may accelerate AD (Zhu et al.).

In all, the gut microbiome can impact AD through numerous overlapping paths rather than one singular mechanism (Loh et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Neuroinflammation can affect amyloid and tau pathology, microbial metabolites can regulate microglial activity, blood-brain barrier dysfunction can expose the brain to peripheral inflammatory signals, and aging can intensify each of these factors (Loh et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Current evidence supports the concept of the gut microbiome being an impactful factor in AD progression, although more human studies are necessary to consider this notion as biologically plausible(Loh et al.; Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies") .

4. Therapeutic Implications and Limitations

4.1 Potential Interventions

There is a need to recognize that microbial dysbiosis poses a significant risk to systemic inflammation and dysregulation of the blood brain barrier as well as amyloid pathology. This has led to sustained scientific interest aimed at targeting microbiome interventions. These intervention attempts can range from dietary and probiotic approaches to fecal microbiota transplantation.

4.1.1 Probiotics / Prebiotics

One microbiome intervention attempt is the probiotic and prebiotic interventions which are generally aimed at restoring microbial balance through enhanced microbial diversity and functional capabilities. This is usually accomplished through the nourishment of beneficial taxa

and some Faecalibacterium species. Possible mechanisms behind this restoration of microbial balance may involve an increased production of short-chain fatty acids to support gut nourishment and reduce inflammation which may also induce neuroprotective benefits via the gut-brain axis. As a result, improved gut-brain barrier function can lead to the reduction of translocation of toxic microbial products that drive inflammation and microglial activation.

Both animal and human evidence appear to support this hypothesis. For example, a systematic review analysing 18 animal studies in Alzheimer disease mouse model showed that selected probiotic formulations can lower the risk of brain amyloid, reduce Proinflammatory cytokines and enhance performance in spatial memory tasks (Naomi et al., 2021). Although human studies are more limited, one early randomized, double-blind clinical trial in Alzheimer disease patients showed that 12 weeks of multiple strains of probiotics produced a modest but statistically significant increase in Mini-Mental State Examination test performance compared with the placebo control group (Akbari et al., 2016). While subsequent trials have noted mixed findings on cognitive and inflammatory markers (Marx et al., 2020; Den et al., 2020), prebiotics can offer complementary results by preferentially feeding indigenous beneficial bacteria.

4.1.2 Dietary Interventions

Diet can be neuroprotective of gut inflammation by acting as one of the dominant modifiers of gut microbial composition. For example, an observational study on mediterranean diet, comprising of high amounts of vegetables, fruits, whole grains, nuts, olive oil and fish consumption, was found to be associated with higher microbial diversity, enriched short-chain fatty acids producing taxa and reduced risks of cognitive decline and Alzheimer's disease (Solch et al., 2022; Dissanayaka et al., 2024).

The mechanism behind the modulating gut microbiota by mediterranean diet can be traced to the high fiber content which enhances the production of short-chain fatty acid while polyphenols from diets like olive oil, vegetables can mimic prebiotic effects and releases direct antioxidant and anti-inflammatory properties

In a randomized, double-blind pilot study, a modified Mediterranean-ketogenic diet was shown to alter the gut microbiome biodata of adults at risk of Alzheimer disease with favourable changes in cerebrospinal fluid biomarkers of Alzheimer disease (Nagpal et al., 2019).

4.1.3 Fecal Microbiota Transplantation (FMT)

Fecal Microbiota transplantation involves the transfer of microbiota from a healthy donor, aimed at reconstituting a healthy balanced microbial community in the recipient. In preclinical transgenic mouse studies, fecal microbiota transplantation from wild specific mice improved cognitive deficits and reduced cerebral amyloid-β deposition (Sun et al., 2019).

Similar neuroprotective effects have been observed in Alzheimer disease models including improved cognitive functions and reduced amyloid pathology. Conversely, the transfer of microbiota from an elderly, Alzheimer Disease animal to a younger or disease free recipient accelerated pathology and cognitive declines (Xiang et al., 2023).

Insight from human studies is limited, and for now, remain only experimental (Ren et al., 2025). A few of those studies in adult patients with mild cognitive impairment or Alzheimer disease have noted temporary cognitive improvements but more clinically, randomized controlled trials are warranted before any solid conclusion can be inferred about its translational potentials in humans.

4.2 Evidence Summary

Insights from animal models that used germ-free rearing, antibiotic-mediated depletion and reciprocal FMT experiments highlight the role of gut microbiota on amyloid plaque load, microglial activation, tau phosphorylation and cognitive performance in mice.

Causality in human studies has been far more limited and empirical evidence has only emerged from observational studies that showed consistent evidence of reduced microbial diversity and compositional differences in Alzheimer disease patients compared to healthy controls (Vogt et al., 2017). While these differences often correlate with the severity of clinical symptoms, yet, the observational nature of these studies leaves the possibility that dysbiosis is a result of Alzheimer disease related changes in diet, medication or inflammation rather than the primary driver of the disease.

4.3 Limitations

To date, some important limitations hinder translational interpretations. Firstly, almost all human evidence is correlational in nature, limiting causality. Interpretations are also constrained by confounding factors such as age, diet quality, polypharmacy, physical activity and other related comorbid metabolic disorders. There is also limited longitudinal research sampling microbiome with the mechanistic biomarker or cognitive trajectories which would have provided insight into the temporal dynamics or critical timelines for interventions.

Conclusion

The gut microbiome likely contributes to Alzheimer's disease progression through immune, metabolic, and neuroinflammatory pathways, but current evidence remains insufficient to establish causality (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Alterations in the composition of microbes can influence microbial metabolite production, blood-brain barrier integrity, peripheral inflammation, and immune activity within the brain (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). These processes can impact the amyloid-beta accumulation, synaptic dysfunction, and tau pathology, (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). However, current evidence does not prove that gut dysbiosis truthfully causes AD, many recent findings are based on animal models or observational studies, thereby leaving researchers with little to no knowledge regarding the factors of AD outside of medication, age, diet, or lifestyle (Seo and Holtzman, "Current Understanding of the Alzheimer's

Disease-Associated Microbiome and Therapeutic Strategies"). Due to this, the gut microbiome is known as a potential contributing factor rather than the definitive cause of AD (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies").

Future research must manage these limitations through intensive human studies that follow a plethora of different participants, from normal aging to mild cognitive impairment to AD (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Studies regarding the mechanistic aspect of AD should also take place to showcase how certain bacteria, their products, and metabolites affect tau phosphorylation, amyloid-beta deposition, microglial activation, neuronal health, and overall systemic inflammation (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Because oftentimes studies report inconsistent bacterial patterns, researchers should examine microbial functions and metabolic activity rather than tracking the bacterial abundance (Seo and Holtzman, "Current Understanding of the Alzheimer's Disease-Associated Microbiome and Therapeutic Strategies"). Another important direction is the integration of microbiome information with neuroimaging, genetics, cognitive testing, and blood biomarkers. AD develops through several biological and environmental factors, and the microbiome's effects can thereby significantly vary depending on the model subject (Zhan et al.). Merging these different forms of data can assist researchers with identifying which individuals have a higher chance of experiencing microbiome-related effects, and thereby also support a more individualized understanding of AD risk and progression in the future (Zhan et al.).

Once definitive proof is available, microbiome research can have important clinical applications(Zhan et al.; T. Zhang et al.). Microbiome-derived metabolites can contribute to variants of biomarker systems for the recognition of AD-related changes, or identifying which individuals have a higher risk of experiencing disease progression (Zhan et al.). Treatments designed to support gut health may also be explored as additions to previously established prevention/treatment tactics (Zhan et al.; T. Zhang et al.). Regardless, these tactics should not be established as clinically validated AD treatments (Zhan et al.; T. Zhang et al.). Individual microbiomes vary greatly, therefore also causing numerous different treatment responses to be effective (T. Zhang et al.). Closely monitored human trials are necessary to deem safety, dosage, long-term effects, as well as which patients are the most likely to benefit from certain treatments (T. Zhang et al.).

In conclusion, studying the gut-brain axis expands the concept of AD far past just a brain-centered model, it supports a larger framework in which the nervous, immune, digestive, and metabolic systems may all be impactful. Even though uncertainty remains, a deeper conceptual understanding of these bodily-relationships can improve research knowledge of AD's mechanisms, and therefore also contribute to earlier detection, supportive therapies/treatments, as well as personalized risk assessments. The gut microbiome is unlikely to provide a simple explanation for AD, but it can play a large role in the systemic approach to neurodegeneration .

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The PANC Paradigm: Analyzing Mindfulness Meditation as a Protective Neurocardiovascular Stress Remediation Mechanism

By Sameera Rampertab and Isabella Cabrera

Abstract

Stress and anxiety have bombarded the lives of teenagers across the world, disproportionately impacting adolescent females. Today's youth continuously drown in the pressures of maintaining high grades, perfect standardized test scores, impressive leadership positions and the daunting college application process, leading to a phenomenon called stress-stacking. Therefore, this study aids in providing an effective intervention to mitigate the virulent neurocardiovascular effects of stress through leveraging the protective effects of mindfulness meditation as a buffer against chronic stress. Journaling and mindfulness meditation were compared to determine which method yielded a higher efficacy of reducing symptoms of stress: increased heart rate, increased blood pressure, decreased theta waves, decreased memory. Previous studies have not addressed this mechanism through the lens of a fast-acting sympathetic deactivation system, reducing the risk of chronic stress. Therefore, there remains a lack of evidence regarding non-clinical interventions that improve specifically neurocardiovascular stress symptoms–which would specifically hold value for adolescent females given the disproportionate impact. This study utilizes the PANC Paradigm for elucidating the protective effects of mindfulness meditation: its release of frontal midline theta waves to activate the parasympathetic nervous system (PNS), stimulate vagal stimulation and release of acetylcholine (ACh), and ameliorate the neurocardiovascular symptoms and biomarkers of stress. A total of 120 trials were conducted, directly testing for the aforementioned symptoms while comparing a) journaling to meditation, b) journaling and meditation to exercise, and c) journaling and meditation to a control group. The SereniBrain EEG Headband and OMRON blood pressure monitor were used for data collection while participants engaged in a series of tests which included memorizing words and writing them down to examine the interventions' effects on memory. The data concluded that meditation produced the most robust results in lowering blood pressure and heart rate when compared to journaling while increasing both memory theta waves during all three phases of the trials. Thus, the practice of mindfulness meditation can serve as a pragmatic approach for reducing neurocardiovascular biomarkers and symptoms of stress, ultimately, providing relief during moments of tension and potentially precluding escalation into anxiety or depression in female adolescents.

Introduction

Stress and anxiety have bombarded the lives of teenagers across the world. In more recent years, both boys and girls have reported significantly higher levels of socially prescribed perfectionism—the belief that peers, parents, and institutions demand unrealistic expectations from them (Curran & Hill, 2019). More specifically, youth in high-achieving school districts experience elevated rates of anxiety, depression, and stress symptoms in the midst of mounting

expectations to embrace perfectionism (Leonard et al., 2015). Additionally, high school students today are confronted with the relentless pressure to cultivate competitive college profiles, stacking their resumes with extensive extracurricular activities and awards. This meritocratic pressure and hyper-competitive environment transform a typical high school experience into one where students drown in chronic psychological and physiological burnout. However, according to the American Institute of Stress, over 54% of teen girls report feeling stressed daily, compared to 43% of teen boys, highlighting a significant disparity. Despite many strategies to mitigate the detrimental effects of stress and anxiety on female adolescents, there is a relative paucity of evidence determining which intervention can be rendered most effective at lowering stress biomarkers. Likewise, confusion remains regarding when meditation and journaling should be employed, especially since journaling is also highly recommended for individuals dealing with trauma.

Anxiety and stress deleteriously impact the brain psychologically and physiologically. For instance, studies have revealed a link between anxiety and increased activation of neurons in the amygdala, the region of the brain that processes fear. During stressful situations, the amygdala disables the prefrontal cortex, which is responsible for executive functioning, emotional control, and reasoning. Additionally, stress provokes the nervous system to flood the body with adrenaline and cortisol, the stress hormones. Accordingly, this eventually leads to dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, the body's stress response system, weakening the immune system and disrupting sleep. Similarly, the hippocampus, essential for memory formation, may shrink or become damaged in the presence of excess cortisol. When considering the biological impacts, it is important to note three major alterations: impaired working memory, decreased emotional regulation, and negative identity development. During an anxiety-driven state, it becomes difficult for the brain to form memories, concentrate, or recall previously learned information. Teens struggle to regulate intense emotions and fail to adapt to perceived "threats," locking their brains in a cycle of constant nervousness. Likewise, stress and anxiety severely compromise self-concept, instilling feelings of inadequacy, fear, and low self-esteem into one's identity. These aforementioned factors ultimately lead to dampened academic performance, addictive behaviors, drug or alcohol abuse, depression, and even suicide if left unregulated. Both mindfulness meditation and journaling have emerged as two promising interventions for calming anxiety by mitigating its symptoms and biomarkers. However, although journaling creates a space for participants to release their thoughts, it may not serve as the most efficient method for quelling the sympathetic nervous system or activating parasympathetic tone. Meditation and journaling differ in three distinct areas: primary pathway, dominant brain waves, and autonomic impact. Meditation is considered a bottom-up intervention, beginning with individual somatic components and specific details before focusing on the larger cognitive picture. This alters the somatic state by enforcing deep diaphragmatic breathing and thereby stimulating the vagus nerve. Furthermore, meditation releases frontal midline theta waves and alpha waves to synchronously produce a calming effect and induce direct parasympathetic activation. Conversely, journaling employs a top-down approach, often

addressing big-picture thoughts, symptoms, and emotions without targeting specific neurocardiovascular mechanisms. In addition, during journaling, theta waves are generated primarily by the hippocampus via Type 2 or cholinergic theta pathways and prefrontal working memory networks. These waves co-fire with beta waves as the brain extracts past memories, feelings, and vocabulary to translate them into written sentences. When journaling, the brain remains actively engaged in analysis and fine motor control, which slows its physiological calming response.

Moreover, journaling focuses primarily on stabilizing mental health rather than directly targeting neurocardiovascular biomarkers of stress and anxiety (Sohal et al., 2022). Meditation, however, aims to relieve tension in both the body and mind. Evidence alludes to Cognitive Behavioral Therapy (CBT) as a method maintaining substantial efficacy in diminishing symptoms of stress and anxiety. As a talk therapy, CBT allows patients to identify patterns among their thoughts, behaviors, and emotions, stimulating positive behavioral changes and emotional management (Nakao et al., 2021). Additionally, Progressive Muscle Relaxation (PMR)—a technique involving the tensing and releasing of muscle groups—is an effective non-pharmacological way to relieve stress (Toussaint et al., 2021). Yet, while PMR is widely known for alleviating physical tension, mindfulness meditation yields more potent effects on reducing specific neurocardiovascular stress symptoms. When determining the most effective non-invasive, non-pharmacological technique for relieving anxiety and stress, evaluating the target areas of these interventions is vital for selecting an optimal protocol. Nonetheless, mechanistic evaluations reveal that journaling may be better suited for trauma recovery than rapid anxiety alleviation. This distinction sparked the central research question: which method maintains a higher level of effectiveness in mitigating neurocardiovascular biomarkers and symptoms of anxiety and stress? Thus, the present study aims to shed light on home-based remediation techniques that spark favorable trends in neurocardiovascular biometrics without requiring formal participant training.

Although journaling and meditation both produce neurocardiovascular protective effects, a rapid-onset intervention is most optimal for mitigating adolescent stress. If meditation is practiced during the college application process or prior to stressful events—such as exams, athletic events, or competitions—the risk of teens becoming overwhelmed through "stress stacking" or long task lists may be partially mitigated (Almadhor et al., 2023). During these high-stress moments, a rapid-onset solution to activate the parasympathetic nervous system is necessary to protect the adolescent nervous system from the detrimental effects of chronic stress. Neurological and cardiovascular correlates of chronic stress—namely decreased theta power, impaired memory, increased heart rate, and elevated blood pressure—indicate systemic autonomic imbalance (Vaccarino & Bremner, 2024). Thus, mindfulness meditation and journaling were identified as two non-clinical, non-invasive mechanisms to reduce these hallmarks of stress.

By comparing both techniques, this study sought to confirm which approach serves as a more efficacious strategy for reducing stress biomarkers and symptoms. Therefore, by

synthesizing the benefits of both interventions with the physiological stress response, the PANC Paradigm (Parasympathetic, Acetylcholine, Neurologic, Cardiovascular) was devised as the primary framework for this investigation. The model determines how parasympathetic stimulation prompts the vagus nerve to release acetylcholine and evoke favorable neurocardiovascular biometric trends. Specifically, the PANC Paradigm posits that activation of the parasympathetic nervous system ("rest and digest") induces theta wave activity, which stimulates the vagus nerve to release acetylcholine and prompt these favorable responses. By combining neuroelectrophysiological and cardiovascular measures, this study aims to elucidate how mindfulness meditation functions as a fast-acting somatic intervention to maintain homeostasis through autonomic modulation. More specifically, these findings provide an evidence-based, non-pharmacological buffer against the disastrous biological impacts of chronic stress in female adolescents.

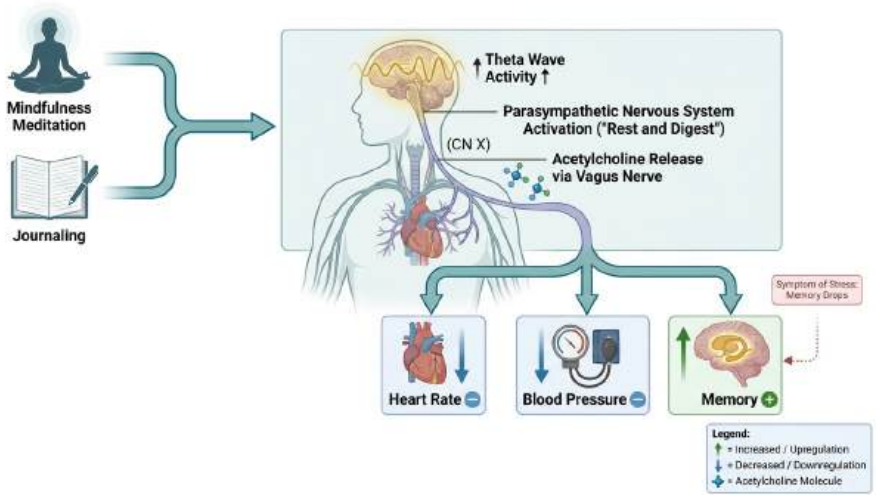


Fig 1: The PANC Paradigm and Biological Stress Mechanisms

Literature Review

Physiological Protective Effects of Mindfulness Meditation

During mindfulness meditation, cortical activity shifts from high-frequency beta oscillations to slower frontal midline theta (fmθ) within the anterior cingulate cortex (ACC) and medial prefrontal cortex(mPFC). This shift reflects heightened internal monitoring and increased parasympathetic regulation driven by the vagus nerve (Cranial Nerve X).Upon activation, vagal postganglionic fibers release acetylcholine (ACh) onto cardiac M2 muscarinic receptors at the sinoatrial (SA) node (Levine et al., 2017 and Newberg and Iverson, 2003). This opens potassium channels and slows SA node firing—directly driving key cardiovascular stress biometrics, including reduced heart rate and elevated heart rate variability (HRV). In contrast, while hippocampal theta (observed during tasks like journaling) also involves ACh for memory processing, it lacks this direct vagal link to cardiovascular control.Because acetylcholinesterase

degrades ACh in milliseconds, direct central measurement in humans via blood, urine, or non-invasive neuroimaging is impossible. Invasive methods like microdialysis are restricted to animal models, leaving human research reliant on predictive neurochemical modeling and functional brain mapping. Moreover, previous studies have "reported improved functions associated with the prefrontal cortex with increased meditation (e.g., attention and working memory)". Similarly, several other brain regions have been positively impacted by the practice of meditation, namely the hippocampus, anterior and mid cingulate, orbitofrontal cortex and corpus callosum. These structures have seen an increase in memory consolidation, memory reconsolidation, emotional regulation and enhanced communication across brain regions (Fox et al., 2014).

Physiological Protective Effects of Journaling

In contrast, mindfulness meditation increases parasympathetic activity and vagally regulated cardiovascular activity; however, journaling engages a variety of neural systems typically used for memory retrieval and for higher-order executive function. For example, during journaling, hippocampal theta oscillations are generated through cholinergic input from the medial septum to the hippocampus. These oscillations are found specifically in the CA1 and Dentate Gyrus regions of the hippocampus, where ACh supports memory encoding and neural interference suppression. Activation of the dorsolateral prefrontal cortex (dIPFC) supports working memory and language planning during the journaling process (Klein and Boals, 2001). Typically, hippocampal theta activity is accompanied by high-beta oscillations in cortical language networks. Such high-beta activity has been hypothesized to reflect sustained analytical processing, such as sentence construction. Meditation-induced frontal midline theta is associated with parasympathetic activity and improvements in cardiovascular indicators such as heart rate variability,

Blood Pressure and Memory

Cortisol plays an important role in linking blood pressure regulation and memory. It is the end product of the hypothalamic-pituitary-adrenal axis (HPA) which activates in response to stress (McEwen, 2007). Cortisol causes an increase in vascular responsiveness and activation of the sympathetic nervous system (McEwen, 2007). Sustained exposure to cortisol can trigger negative effects on the cardiovascular as well as the neurological system (de Kloet et al., 2005;Lupien et al., 2009). For example, hypertension and impaired hippocampal synaptic plasticity, which can cause reduced memory (Iadecola & Gottesman, 2019; Lupien et al., 2009). The consistent activation of the HPA axis provides a common biological pathway which contributes to both hypertension and memory impairment (de Kloet et al., 2005; McEwen, 2007).

Heart Rate and Memory

Heart rate is a marker of cognitive function. This is due to the relationship between heart rate and the nervous system (Thayer et al., 2012). Increased heart rate is caused by the activation

of the sympathetic nervous system which releases stress hormones like cortisol and catecholamines (McEwen, 2007). Although these changes are adaptive for a short period of time, sustained heart rate elevations are associated with declining cognitive function (Thayer et al., 2012). Previous studies have found that impaired memory and executive function are connected to elevated heart rates (Thayer et al., 2012). This is caused by autonomic imbalance with higher sympathetic activity and lower parasympathetic tone which has been associated with lower heart rate variability (HRV), a measure consistently linked with poorer memory, executive functions, and cognitive flexibility (Shaffer & Ginsberg, 2017; Thayer at al., 2012).

Theta Waves and Memory

Theta waves are vital for increasing explicit memory–the conscious, intentional recollection of factual information, concepts, and previous experiences. Magnetoencephalography (MEG) and EEG studies have collectively observed an increase in theta waves during mindfulness meditation, "overlying frontal regions" (Wong et al., 2015). This phenomenon can also be explained by the fact that many studies have found increased recall with handwriting than typing. Writing increases the amount of synchronized theta rhythms associated with memory consolidation (Marano et al., 2025). Low theta frequency waves (3-7 Hz) have been linked to emotional memory, encoding and the hippocampus. These waves act as "glue", binding different sensory inputs into long-term memories. Similarly, high frequency theta waves (8-12 Hz) are tied to the prefrontal cortex and working memory for an increase of information processing. Both processes are connected to cognitive capacity and enhance neuroplasticity. For a memory to form, synapses or neuron communication sites must be strengthened through a process called Long Term Potentiation (LTP). Neuronal synapses become highly receptive to LTP when theta waves are active. Long term potentiation acts as a "save button" instructing the brain to save the memories and store them (Smolen, 2007). Additionally, the hippocampus, or part of the brain where memories are stored and prefrontal cortex, responsible for decision making and executive function must communicate during memory formation and recollection (Preston & Eichenbaum, 2013). Theta waves fire in synchrony when mindfulness meditation is practiced, enhancing communication between both neurological structures to enhance memory recollection and learning. During an environment where theta waves are predominant, cognitive noise is eliminated, allowing memory of a formation and recollection to be more focused. Neurons also experience "phase locking" when fired at the trough of a theta wave, heightening the electrical signal (Guth et al., 2024). Likewise, at the peak of a theta wave, the efficiency of memory retrieval from the hippocampus increases.

Theta Waves, Cortisol and Adrenaline

Cortisol and adrenaline, two stress hormones, maintain an inverse relationship with theta waves. Theta waves suppress these stress chemicals and therefore, the sympathetic nervous system responsible for the fight-or-flight response. The hypothalamic-pituitary-adrenal (HPA) axis is the body's primary neuroendocrine stress response system, driven by the activation of

both cortisol and adrenaline (Sheng et al., 2021). Specifically, frontal midline theta waves, waves often seen during mindfulness meditation, quiet the neuronal firing in the amygdala and deactivate the HPA (Tang et al., 2019). Adrenaline receptors stop binding to the heart, decelerating mechanisms that produce high blood pressure and heart rate. Adrenaline acts fast and is also cleared fast, whereas cortisol lingers for a longer duration of time and becomes toxic to the hippocampus (Knezevic et al., 2023). Since theta waves suppress cortisol, it removes strain on neural networks for learning, allowing for an increased genesis of synapses. When cortisol and adrenaline become dominant, this triggers an excess release of glutamate, an excitatory neurotransmitter that is a biomarker of stress and increased neuronal firing. Although this neuron firing constitutes heightened brain activity, theta waves are associated with triggering the release of GABA (gamma-aminobutyric acid), an inhibitory neurotransmitter that binds to neurons. This neurotransmitter acts as a shield, precluding rapid firing commonly associated with a stressed state. Additionally, the brain-derived neurotrophic factor (BDNF) is also suppressed by excessive levels of cortisol (Binder & Scharfman, 2004. This protein promotes the growth of neurons (neurogenesis), strengthening synaptic connections and ensuring an enhancement of neuroplasticity. As levels of GABA (gamma-aminobutyric acid), an inhibitory neurotransmitter, and theta waves increase once again, this reinforces cellular balance. During stressful states, the blood's pH is often altered by the changing amounts of oxygen and carbon dioxide triggered by different respiration rates. This change in pH sends a signal to the vagus nerve to instate a positive feedback loop. Theta waves lead to less stress, relaxation, and therefore, an increase in theta waves (Capilupi et al., 2020). Adrenaline's counterpart, norepinephrine, the neurotransmitter lingering in the brain, exists predominantly in the locus coeruleus which increases the amount of firing in this part. Theta waves suppress this firing, leading to a more relaxed state and triggering the parasympathetic nervous system.

Parasympathetic vs. Sympathetic Nervous System

Both the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS) are branches of the autonomic nervous system. The parasympathetic nervous system acts as the body's rest-and-digest mechanism, allowing for relaxation, whereas the sympathetic nervous system propels the fight–or-flight system, activating the stress response (Weissman and Mendes, 2021). Heart rate increases during stressful states as a result of the sympathetic nervous system with adrenaline causing the heart to beat faster and harder to pump blood (Tindle and Tadi, 2022). During the restful states when the parasympathetic nervous system dominates, the vagus nerve releases acetylcholine, slowing heart rate. Regarding blood pressure, spikes occur during stressful states, once again as a result of adrenaline increases, narrowing blood vessels (Gordon et al., 2015). Blood pressure drops as adrenaline is suppressed and blood vessels dilate, allowing for the pressure to decrease during less stressful states. Additionally, theta waves are suppressed when the sympathetic nervous system becomes predominant, as the brain aims to maintain high frequency beta and gamma waves. Nevertheless, these waves increase during relaxation, triggering the suppression of high frequency beta and gamma waves. During stressful

events when cortisol is increased, memory is impaired blocking the formation of long-term memories (Klier & Buratto, 2020). However, during states of low stress, memory is enhanced due to the increase of acetylcholine and attenuation of cortisol.

Hypotheses

Hypothesis I. Mindfulness meditation increases memory more than journaling.
Hypothesis II. Mindfulness meditation increases theta waves more than journaling.
Hypothesis III. Mindfulness meditation decreases blood pressure more than journaling.
Hypothesis IV. Mindfulness meditation decreases heart rate more than journaling.

Objectives

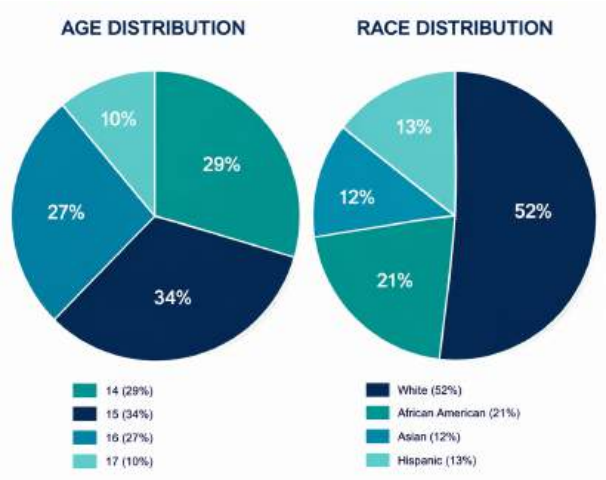
In this study, we intended to:

- Advance the view of the PANC Paradigm to analyze the roles of different theta wave types: frontal midline theta waves and hippocampal theta waves
- Determine how theta waves and memory scores increase due to acetylcholine and if blood pressure and pulse rate will decrease

Sample

After IRB (Institutional Review Board) approval was obtained, the survey was distributed via convenience sampling to support this quasi-experimental study. The intended sample for this research study was female high school students, from a single sex school in the United States. 30 high school females participated in the whole study. Anonymity of the participants was maintained by solely requesting the last 5 digits of their phone number, so that responses could be removed if requested.

Demographics



Measures

Both biomarkers and symptoms were included to provide both objective and subjective data, connecting the mind and body. Biomarkers provide concrete physiological data that cannot be influenced by participant bias. Additionally, symptoms provide subjective psychological evidence, recording acting as a behavioral sensitivity test to support the physiological aspects with cognitive data. Therefore, the inclusion of both subcategories of biometrics adds a more intricate dimension to this study, offering a more holistic approach to validating the PANC Paradigm.

Biomarkers

Theta Waves

Theta waves, 8-12 Hz were measured through the SereniBrain headband's electrodes. These waves were measured specifically to determine which intervention had a greater amount. A greater amount of theta waves would signal a more robust cognitive effect through an enhanced release of the memory-improving neurotransmitter, acetylcholine. An increase in theta waves would indicate a state of deep, relaxed focus, reduced mental noise and heightened cognitive absorbance. Although the theta waves in each intervention are different, testing for the presence of the waves would demonstrate which method would substantiate the PANC Paradigm. A higher amount of theta waves indicates two specific things: a cognitive state categorized by lower brain activity and a state of relaxation.

Heart Rate

Heart rate was measured through the OMRON Blood Pressure monitor in beats per minute (bpm). This metric was obtained specifically to determine which intervention would prove as a fast-acting physiological brake, reducing the activity of the sympathetic nervous system. Since an elevated heart rate serves as a biomarker of stress, this metric indicates an increase of cortisol, adrenaline, and norepinephrine. The intervention which triggered the sharper decrease would be indicative of which method could, mechanistically, hyperpolarize the cells faster, slowing their firing and in turn, reducing heart rate.

Systolic and Diastolic Blood Pressure

Systolic blood pressure was considered the sympathetic "power" marker, measuring the maximum pressure exerted against arterial walls when the heart actively beats. Diastolic blood pressure, the parasympathetic "vascular tone" marker, is the minimum pressure in the arteries when the heart rests between beats. Both types of blood pressure were recorded through the OMRON Blood Pressure Monitor in millimeters of mercury (mmHg). Systolic and diastolic blood pressure were included because they both index physiologic mechanisms imperative to this study; the former measures sympathetic activity, and the latter is indicative of parasympathetic signaling through a decrease.

Memory Levels

Memory levels were measured through a simple memory test consisting of ten words. Participants were given either 1 min or 1 min 30 secs depending on their age to account for the word difficulty since freshmen and seniors maintain different levels of academic strength. They were asked to memorize the ten words in order and write them down after memorizing. Each word was worth ten points, and scores were calculated out of 10 as a percentage of 100. For example, 2/10 would equal 20%. Higher scores indicated a higher level of memory and cognition and vice versa. Memory scores were measured because during times of stress, memory often decreases and becomes clouded. The phase that yielded a more significant increase in memory scores would signify a decrease in cognitive noise and, synchronously, an amplification of focus.

Procedure

To test our hypotheses, the full experiment included three phases after approval was obtained (Fig 2). During Phase 1, single-blind placebo controlled trials were conducted. On day 1, participants were asked to journal for four minutes while the SereniBrain EEG headband recorded the theta waves. Participants were asked to journal about their current thoughts and events during their day. After the four minutes, the OMRON Blood Pressure Monitor was used to measure heart rate and both systolic and diastolic blood pressure. Participants were directed to a memory test where they were instructed to memorize 10 words in order in either 1 minute or 1 minute and 30 seconds depending on age. They were then told to write the words down in order and their score was calculated out of 100. On day 2, participants were asked to meditate for four minutes with the same subsequent procedures. During the next two phases, the trials maintained an open-label design. Theta waves were not measured during the exercise and control trials because of the distinct meanings accompanied by this metric during meditation versus activity. Theta waves naturally spike during periods of activity and would have, therefore, yielded a false positive, diminishing the accuracy of the results. Phase 2 specifically consisted of the control trials. On day 1, a control group was included where participants memorized a list of words for 1 min and 10 secs, mimicking their daily routine in school–absorbing information. Then, the meditation trial was conducted in the same way as Phase 1. However, there was a gap of five minutes allotted before administering the memory test to ensure participants had enough time to recover from any type of cognitive fatigue. On day 2, the control group was followed by the journaling session with the same measurement procedures for blood pressure, heart rate, and memory (a five-minute gap was allotted, similar to day 1 of Phase 2. Furthermore, during Phase 3, the exercise trials occurred where participants were instructed to walk at a fast pace for 1 minute and 30 seconds. They were instructed to walk across a classroom 15 times. On day 1, subsequent to the exercise trial, the meditation session took place with the same procedures for measuring blood pressure, heart rate and memory, yet the five-minute gap was removed. On day 2, the journaling trial occurred subsequent to the exercise trial with the same subsequent procedures. Additionally, control trial was implemented as a neutral baseline of comparison. If heart rate or memory scores were solely measured after meditation, this trial would be unable to

display the neurocardiovascular protective effects of mindfulness meditation. By introducing a neutral control group, this phase allows for the observation of a true effect of how meditation works in everyday life: enhancing memory while lowering cardiovascular metrics subsequent to active periods. The exercise trial served as an acute physiological stressor designed to intentionally overactivate the sympathetic nervous system. More specifically, this was incorporated to deliberately increase heart rate, blood pressure and suppress acetylcholine, enabling us to test the recovery power of both interventions. The inclusion of both Phase 2 and Phase 3 was methodologically essential to isolate the mechanisms of recovery from stress. During the statistical analysis, the scores of Day 1 and Day 2 for the exercise and control groups were averaged to create a composite control, dampening day-to-day biological noise. Participants are often slightly anxious on Day 1 due to the new equipment and conscious presence of the researcher. In addition, averaging the scores of both days serves as the exercise score for heart rate, blood pressure, and memory or the control score for heart rate, blood pressure and memory may also rule out this novelty effect which also accounts for sleep quality or background noise. This choice was purposeful in providing a highly stable and accurate physiological representation of the participants' baseline and stressed state against which the effects of both interventions could be evaluated. A series of four paired t-tests were used to determine whether mindfulness meditation or journaling would ultimately yield a significant difference in the increase of neurological metrics (theta waves and memory) while decreasing cardiovascular metrics (heart rate and blood pressure). Additionally, a one-way ANOVA for the control and exercise groups regarding heart rate, blood pressure, and memory scores. Initially, we compared the control to meditation and journaling. First, memory scores after the control trial were compared to the memory scores of both the meditation and journaling trial to determine which intervention yielded a more prominent score increase. This same analysis was repeated with the heart rate and blood pressure metrics to determine which technique generated a sharper drop in cardiovascular biomarkers. Next, the exercise trials were compared. The three dependent variables of memory scores, heart rate and blood pressure were compared to the two interventions, each in three separate analyses with a method equivalent to the aforementioned control phase. Statistical significance was obtained through collecting p values less than 0.05.

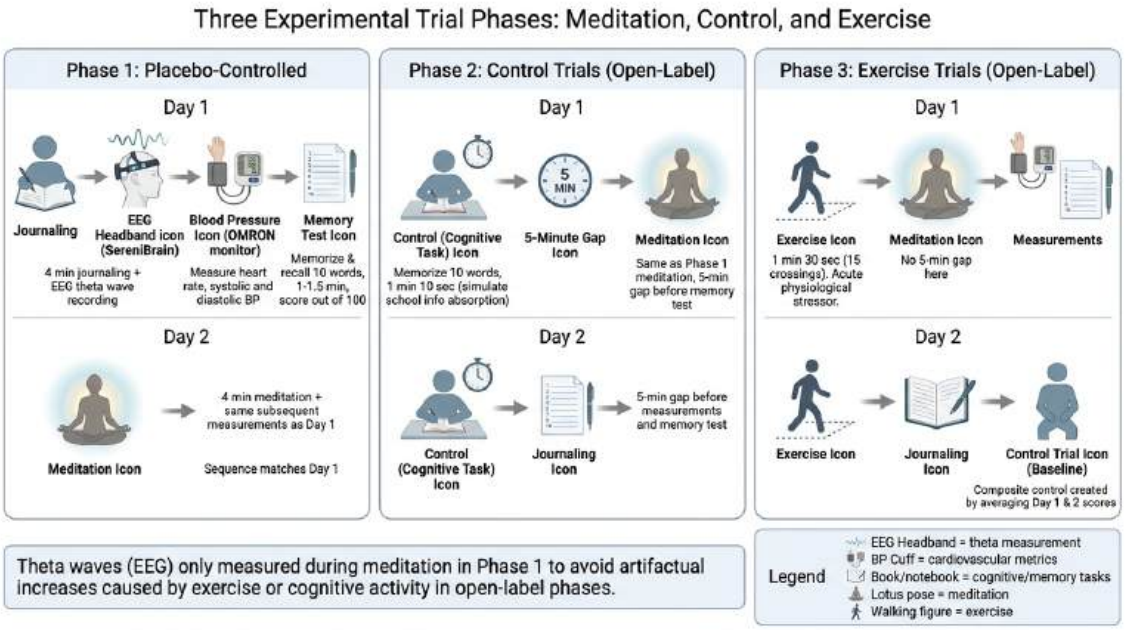


Fig 2: All Three Phases of Experimentation

Results

Statistical significance for all analyses was set at $\alpha = 0.05$. Phase one evaluated differences between journaling and meditation. Phase one utilized a series of four paired t-tests to evaluate the differences between journaling and mediation. For phase two, a one-way ANOVA for the control and exercise groups regarding heart rate, blood pressure, and memory scores were conducted.

Theta Waves

Participants exhibited a statistically significant 13.3% higher mean percentage of theta wave activity during meditation (45.3% ± 5.80%) compared to journaling (39.7% ± 9.82%) (p=0.00525) (Fig. 3).

Memory Scores

Meditation produced a statistically significant +13% increase in the mean memory score (95.0 ± 4.83%) compared to the journaling score (84.28 ± 9.64%) (p= 0.03023) (Fig. 3).

Blood Pressure

Meditation resulted in a significantly lower mean diastolic blood pressure (68.9 mmHg) than journaling (72.4 mmHg) which represented a -0.05% relative reduction (p= 0.02815). For systolic blood pressure meditation had a significantly lower mean blood pressure (105.2 mmHg) than journaling (111.5 mmHg), which represented a -0.56% relative reduction (p= 0.04121) (Fig. 3).

Pulse Rate

The mean pulse rate was lower during meditation (76.48 bpm) compared to journaling (80.24 bpm) however the decrease was not statistically significant (p= 0.0756) (Fig. 3).



Fig 3: Phase 1 Data (Journaling vs. Meditation)

Exercise vs. Meditation vs. Journaling

When comparing exercise to meditation to journaling using ANOVA tests, all variables showed significant differences across all the activities. Memory scores varied across all conditions (p = 0.00024 for exercise vs. meditation) with meditation (92) yielding higher performance than exercise (72.5) and journaling (78). Exercise produced the highest systolic blood pressure (134 mmHg) which was significantly greater than both meditation (103 mmHg p = 0.002) and journaling (110 mmHg p = 0.125). For diastolic blood pressure exercise (83 mmHg) was significantly higher than both meditation (68 mmHg, p = 0.014) and journaling (73 mmHg, p = 0.047). For pulse rate, the difference between exercise (84.5 bpm) and meditation (77.5 bpm) was statistically significant (p = 0.0102), while the comparison between exercise and journaling (77.9 bpm) was not (p = 0.053) (Figs 4-6).

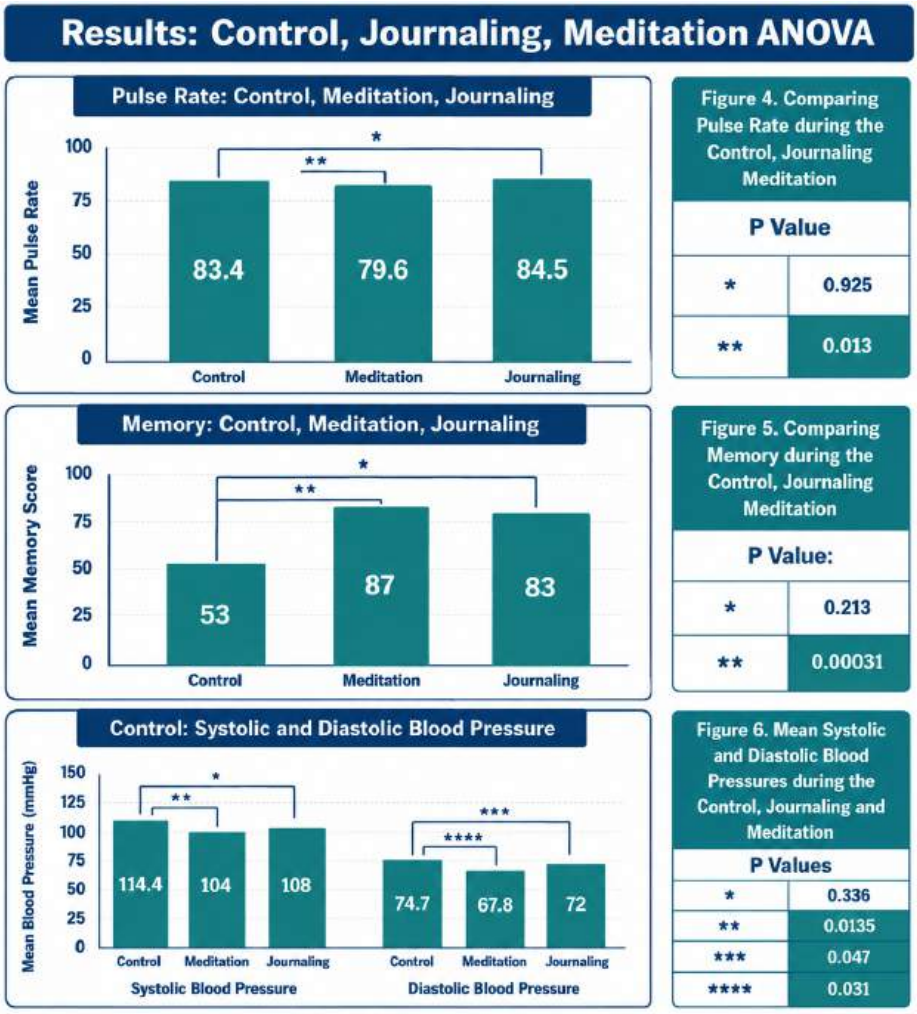
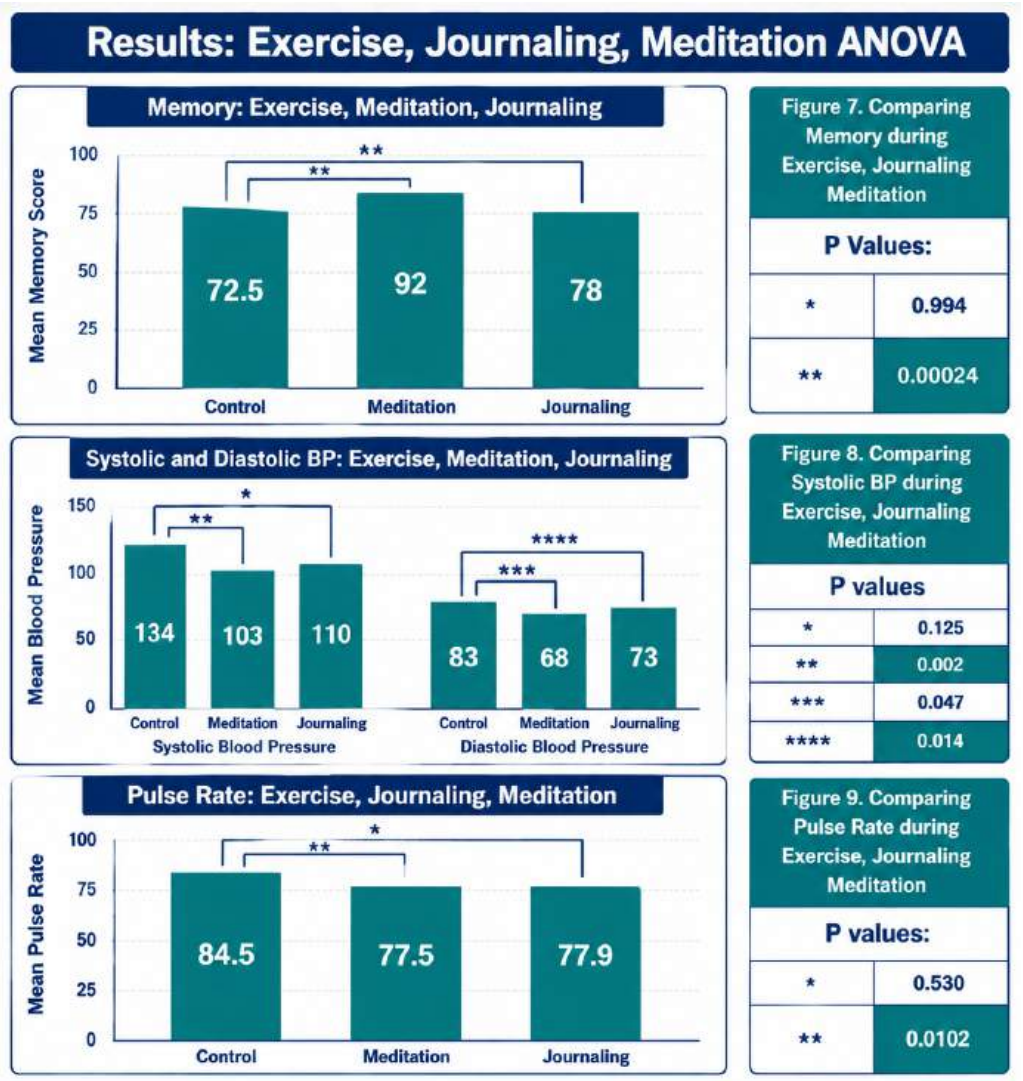


Fig 4-6: Exercise and Meditation Compared to the Control

Control vs. Meditation vs. Journaling

When comparing the control group to meditation and journaling utilizing ANOVA tests, meditation consistently had a higher statistical significance than journaling. Meditation had the highest memory scores (87) when compared to the control (53, $p = 0.00031$). Journaling (83) compared to the control was not as statistically significant ($p = 0.213$). For systolic pressure, meditation had the lowest mean (104 mmHg) when compared to the control (114.4 mmHg, $p = 0.0135$). Journaling (108 mmHg) against the control was once again not as statistically significant ($p = 0.336$). The results for diastolic blood pressure follow the same trend as systolic pressure, with meditation having the lowest mean (67.8 mmHg) and greatest statistical significance in comparison with the control (74.7, $p = 0.031$). Journaling had a lower diastolic blood pressure (72) than the control but was not as statistically significant ($p = 0.047$). For pulse rate, memory once again had the lowest pulse rate (79.6) and the greatest statistically significant

difference to the control (83.4 bpm, p = 0.013). Journaling instead had a higher pulse rate than the control (84.5, p = 0.925) (Figs 7-9).



Figs 7-9: Exercise and Meditation Compared to the Control

Discussion

To test whether mindfulness meditation or journaling causes a bigger jump in theta waves, we used the SereniBrain EEG headband to track brain activity after both activities. As predicted, meditation produced significantly higher frontal midline theta waves than journaling ($p < 0.05$). This increase is crucial because it validates the PANC Paradigm as a key reason why meditation outperforms journaling for stress relief. While journaling stimulates hippocampal theta waves—releasing acetylcholine (ACh) to boost memory—it doesn't offer cardiovascular

benefits. Meditation, on the other hand, prompts the vagus nerve to flood the heart's sinoatrial (SA) node with ACh. This hits the brakes on the sympathetic nervous system within seconds, preventing prolonged cardiovascular stress. For adolescents facing stacked daily stressors, this rapid parasympathetic activation is vital for lowering chronic stress risks. Interestingly, deeper or longer meditation sessions can shift brain activity toward alpha waves while reducing theta waves. While alpha waves don't trigger vagal stimulation, the theta surge during early or short-term meditation is more than enough to drive positive neurocardiovascular benefits. This link between meditation duration and brainwave states aligns with research by Katyal and Goldin (2024) and offers a practical framework for guiding adolescent practice.

Our second hypothesis explored whether mindfulness meditation improves memory more than journaling. After meditating, participants scored significantly higher on memory tests compared to when they journaled. Meditation also showed a statistically significant improvement over both the control and exercise groups, whereas journaling failed to reach statistical significance against either. Still, average scores after journaling were 56.60% and 7.59% higher than control and exercise baselines, respectively—aligning with past research showing that writing about deep thoughts can boost working memory capacity. On a mechanical level, meditation enhances memory by regulating the HPA axis alongside Long-Term Potentiation (LTP). Academic or physical stress triggers the HPA axis to flood the brain with cortisol, which impairs synaptic plasticity and disrupts hippocampal memory consolidation. Meditation acts as a physiological brake, stimulating the vagus nerve to suppress HPA activity and shield hippocampal neurons from stress damage. Journaling, by contrast, relies heavily on the left dorsolateral prefrontal cortex (dlPFC) for language and fine motor tasks, leading to cognitive fatigue before the HPA axis can cool down.These results mirror findings by Basso et al. (2019), who demonstrated that mindfulness meditation significantly boosts working memory accuracy ($p = 0.002$). Beyond helping students clear cognitive clutter before exams, meditation's ability to increase acetylcholine suggests it could also serve as an accessible, non-invasive therapy to help slow cognitive decline in Alzheimer's patients.

When examining blood pressure as a dependent variable, mindfulness meditation consistently drove significant reductions in both systolic (SBP) and diastolic (DBP) blood pressure across all three phases. During Phase 1, meditation lowered SBP and DBP by triggering frontal midline theta waves, which stimulate the vagus nerve to release acetylcholine (ACh) and induce nitric oxide vasodilation. In Phase 2, meditation outperformed journaling, as vagal ACh release actively reduced vasoconstriction to keep arterial walls supple. By Phase 3, meditation again yielded significantly lower blood pressure following exercise, whereas journaling did not, successfully confirming Hypothesis 3. These results align directly with the PANC Paradigm. Meditation acts as a fast-acting somatic brake, leveraging vagal ACh to rapidly switch on the parasympathetic nervous system and cut off the stress response. Journaling, on the other hand, is a top-down cognitive process that consumes working memory for language processing without directly stimulating the vagus nerve (McGuire et al., 2005). These physiological patterns echo previous research by Agrawal et al., who also observed concurrent increases in theta and alpha

EEG activity during meditation, as well as Goldstein et al., who noted that mindfulness-based stress reduction (MBSR) can yield clinically meaningful reductions in blood pressure (Goldstein et al., 2012 and Agarwal et al., 2025). While findings from the HARMONY trial showed that meditation didn't significantly reduce blood pressure in unmedicated Stage 1 hypertensive patients over eight weeks, this highlights a critical distinction: meditation may be most effective as an immediate cardiovascular buffer after acute stress rather than a standalone cure for chronic hypertension (Blom et al., 2012). Ultimately, while long-term journaling has shown blood pressure benefits in extended literature—suggesting our short study window may have limited its observed impact—meditation offers a powerful, real-time mechanism to protect the heart against day-to-day stress stacking.

When examining the effects of both interventions on heart rate, the results were considerably more complex. During Phase 1, no significant difference in heart rate was observed between journaling and meditation. This was likely due to baseline biological "noise" or the inherent sensitivity of heart rate as a parameter. However, during Phases 2 and 3—when measured against the control and exercise trials—mindfulness meditation continuously produced significantly lower heart rates than journaling, successfully supporting Hypothesis 4.
While simple sitting and rest during journaling are enough to passively settle heart rate during low-stress conditions (Phase 1), journaling lacks the direct vagal trigger needed to counter active stress. Post-exercise, the sympathetic nervous system leaves lingering norepinephrine and epinephrine (adrenaline) in the bloodstream, keeping the heart's sinoatrial (SA) node firing at an elevated rate. This is where meditation shines: while journaling relies on hippocampal theta waves for memory, meditation releases frontal midline theta waves—the central command signal that stimulates the vagus nerve. This vagal stimulation floods the SA node with ACh, providing a fast-acting "somatic brake" to override elevated pulse rates. Exposing participants to stress in Phases 2 and 3 was crucial for isolating which practice could actively counter cardiovascular strain. These results confirm that while both practices offer quiet rest, meditation is uniquely suited to provide an active physiological brake following acute stress—a finding supported by prior research showing significant heart rate drops following 90-minute meditation sessions in both face-to-face and virtual settings.

Limitations, Implications and Future Directions

While our findings contribute to a growing body of evidence, several limitations warrant consideration. The sample size of 30 female adolescents, though adequate for observing significant effects, was relatively small due to the time-consuming within-subjects design requiring three separate lab visits. Additionally, because the sample was exclusively female, including male participants in future studies would enhance generalizability. EEG data were also limited by our hardware; the SereniBrain headband could not directly differentiate frontal midline theta waves from hippocampal theta waves. Consequently, to explicate the PANC Paradigm, we relied on established literature regarding wave profiles in journaling and

meditation. Advanced connectivity and localized scalp sensors in future research would pinpoint these specific wave dynamics more precisely.

Future studies are merited to fully fathom the unique mechanisms behind both practices. Tracking alpha waves during meditation and beta waves during journaling will be essential for detecting the specific type of theta wave produced in each condition. Furthermore, administering the State-Trait Anxiety Inventory (STAI) before and after trials would establish which intervention is more effective at reducing real-time anxiety. Creating and validating a present-moment stress survey using Cronbach's alpha would similarly strengthen these findings. Integrating these self-reported psychometrics alongside our biometric data will offer a more comprehensive view of the cognitive impacts driving stress reduction.

Although both practices confer preventative benefits against chronic stress, mindfulness meditation demonstrated superior effectiveness in catalyzing favorable neurocardiovascular trends. Meditation serves as a direct catalyst for parasympathetic activation, stimulating the vagus nerve, increasing acetylcholine, and suppressing cortisol and sympathetic tone. In practical terms, it offers an accessible tool for immediate stress relief, anxiety reduction, and enhanced memory and focus during academic tasks. Practiced consistently over time, meditation's regulation of the autonomic nervous system and neurochemistry may also shield against long-term risks associated with hypertension, Alzheimer's disease, and chronic psychological disorders.

Conclusion

This study investigated whether mindfulness meditation or journaling was more effective at improving neurocardiovascular biomarkers associated with stress. Exercise and control conditions were used as comparisons. Mindfulness meditation produced the strongest improvements across cognitive and cardiovascular outcomes. All hypotheses were supported or strongly suggested, with statistically significant p values and meditation outperforming journaling in most measures. These findings support the proposed Parasympathetic Acetylcholine Neurocardiovascular (PANC) Paradigm. Despite journaling demonstrating beneficial effects in memory scores, its effects seem to rely on cognitive processing rather than autonomic regulation elicited by meditation. Journaling did not produce the same magnitude of benefits as meditation did following stress. Thus, these findings suggest that meditation is a more efficient physiological buffer against acute stress, mitigating the immediate effects of sympathetic activation before there is a chance for chronic dysregulation.

The most novel contribution of the present study is the integration of the neurophysiological and cardiovascular biomarkers in one experimental framework. This study examines how changes in theta wave activity correlates with measurable improvements in memory, blood pressure, and heart rate, instead of examining singular outcomes. This provides evidence for an integrated neurocardiovascular mechanism within stress regulation to offer preliminary support for the PANC Paradigm as a model for describing the neuroelectrophysiological mechanisms and the effects of mindfulness meditation on them. Since

the findings remain consistent with other studies reducing the biometrics of stress, meditation may serve as an effective strategy for high school students, especially females, before tests, prior to competitive events and even during the college application process. Combating these ubiquitous pressures of academic stress, extracurricular activities and perfectionism requires the PANC Paradigm's lens to activate the parasympathetic nervous system, aiming to hinder the development of chronic stress. Altogether, this study suggests that mindfulness meditation is a relevant, non-clinical, non-invasive, and accessible intervention method to prevent chronic psychological and physiological stress. It may represent an effective strategy for reducing long-term mental and physical deterioration by combatting the stress of a vulnerable teenage population.

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Attitudes and Perceptions of Pharmacists Towards Artificial Intelligence in Pharmacogenomics By Shreya Shewale

Introduction

Pharmacogenomics (PGx)

Traditionally, methods of testing medicines have relied on broad populations, limiting its effectiveness on specific individuals. To combat this issue, precision medicine has surfaced as an approach to fighting and preventing diseases and medical conditions by taking into account an individual's genetic makeup, environment, and lifestyle [9]. Precision medicine actively addresses the limitations of traditional drug testing by creating treatment methods that are specifically tailored to a patient's need, boosting efficacy. Within this broad field, several subsets have been created to emphasize different aspects of precision medicine, including pharmacodynamics, pharmacokinetics, pharmacogenomics; this paper will focus on the latter. Similar to the definition of precision medicine, pharmacogenomics can be defined as the "study of how genes affect a person's response to drugs" [9]. This type of personalized medicine is commonly used in prescribing medications for serious health conditions—cancer, HIV, and heart disease—because they require extreme precision. Furthermore, pharmacogenomics is a relatively new field, mainly implemented in the research sector. However, rapid development has led to the clinical usage of pharmacogenomics, where medical professionals—especially pharmacists—identify and predict the most optimal drug and drug dosage. Pharmacists are given a special emphasis in this study because they are the professionals who handle medications directly, verifying drug dosages and drug interactions. Pharmacists interpret pharmacogenomic tests, which are the results of a blood or cheek swab that is sent to a lab to check DNA for any changes. Each individual person's genetic makeup is different, and these variations influence drug efficiency, metabolism, and toxicity, explaining the reason why some patients may experience therapeutic success while others may experience treatment failure or harmful side effects, known as adverse reactions. Adverse drug reactions are ranked between the fourth and sixth leading cause of death in the United States of America, with most fatal adverse reactions happening in 0.32% of patients and costing the United Kingdom an economic burden of £380 million [17]. This justifies an urgent need to prevent adverse reactions from occurring. Gobbs explains that predicting drug response and potential toxicities through pharmacogenomics significantly optimizes patient outcomes by reducing the chances of adverse reactions [5].

Although pharmacogenomics contains great potential, there are many barriers preventing the widespread adoption of pharmacogenomics in pharmaceutical and clinical settings, such as the complexity of genetic information, insufficient and inadequate education, costs, and lack of uniform regulation.

Literature Review

History of PGx

PGx can be traced back to the mid twentieth century, where the term

"pharmacogenomics" was developed by Friedrich Vogel, a German pharmacologist. This was after Arno Motuskly wrote a paper establishing that drug reactions could be a model for studying interactions between environment and disease development and heredity [12]. The earliest research was focused mainly on phenotypes, where observable traits were identified and classified, and then related to certain genotypes. In 2003, the Human Genome Project was completed, allowing researchers to have complete access to the entire genome rather than just individual genes [12]. This increased the complexity of genomic data, justifying the need for a tool to ease managing this data.

Artificial Intelligence (AI) in PGx

Due to the complexity of the increasing amount of genomic data, AI has emerged as a potential tool for comprehending and maintaining datasets. AI can be defined as "a transformative force in global healthcare delivery…applied to algorithm-driven drug discovery, automated medication adherence systems and clinical decision support tools" [1]. Large language models (LLMs), a subset of AI, can be used to build chatbots to assist with complex medical specialties, like PGx [7]. Additionally, LLMs can identify patterns within vast datasets, creating a more accurate way to predict the responses of drugs [14]. Combining technology such as this with genetic information will reduce the workload of pharmacists and provide a new viewpoint that would be difficult to see otherwise.

Pharmacists Existing Attitudes Towards PGx

Existing literature supports the idea that pharmacists have a positive outlook towards pharmacogenomics while simultaneously expressing uncertainty about it. Pharmacists expressed that pharmacogenomics was important to patients; however, a very slim portion of pharmacists felt comfortable managing pharmacogenomic test results [17]. Although many doubts are expressed, research indicates a growing preparedness and readiness to use pharmacogenomic tools. A study indicated almost 50% of surveyed pharmacists showed readiness to interpret and engage in pharmacogenomic test results; additionally, almost 80% showed interest in starting pharmacogenomic services immediately [10]. This research indicates that the gap between readiness to use and actual knowledge is due to systematic gaps in education, not a lack of professional interest.

Pharmacists Existing Attitudes Towards AI

Similar to how pharmacists feel towards pharmacogenomics, pharmacists express enthusiasm but also doubt about utilizing AI tools. About 63% of pharmacists were likely or very likely to adopt AI based tools in the future, showing a shift towards accepting such types of supportive technologies [1]. However, they express doubts about patient privacy and safety; healthcare professionals feel uncertainty and hesitation when discussing responsibility and liability regarding AI [16]. Since errors in this field of medicine can result in severe adverse consequences, there is more reluctance to adopt AI in pharmacogenomics.

Gap

This paper will explore the intersection of two bodies of literature: pharmacists' feelings towards PGx and pharmacists' feelings towards AI. Previously, these two job fields have been researched in parallel and no research has been conducted on the combination of the two. There are no studies that specifically describe pharmacist's attitudes, beliefs, perceptions, and readiness to use AI tools in PGx. The gap in knowledge is significant because pharmacists are the main decision makers regarding medicine prescription. The implementation of AI supported tools without the insight of pharmacists may limit effectiveness and efficiency. Furthermore, without data on pharmacists' perceptions, healthcare systems and policy makers do not have adequate evidence that is necessary to create and design these technologies.

Mohamed and colleagues previously discovered that overall beliefs of pharmacists towards pharmacogenomics are positive, with 90.8% of pharmacists expressing positive views towards the incorporation of pharmacogenomics in clinical settings [10]. Additionally, Jarab and peers found that healthcare professionals have positive attitudes towards the use of AI in healthcare [6]. However, healthcare professionals have concerns regarding the accuracy and reliability of AI in such a risky setting, as human lives can be at risk [6].

This raises the question: what are pharmacists' attitudes and perceptions toward the usage of artificial intelligence as an assistive tool for clinical decision making in pharmacogenomics? This study hypothesized that pharmacists will exhibit overall positive attitudes and perceptions toward the use of AI as an assistive tool for clinical decision making in pharmacogenomics. However, pharmacists' will still express hesitancy due to concerns regarding AI.

Methodology

Study Design and Participants

In order to determine the attitudes and perceptions of pharmacists regarding the implementation of AI in PGx, a descriptive, cross sectional, mixed methods study was utilized. The aim of a descriptive methodology is to describe the characteristics and behaviors of a population, as well as identify patterns that occur within the demographic [13]. This methodology establishes foundational knowledge that can inform further research. Several alternative methodologies could have been used for this project, such as the Delphi method. Although this method would be useful for predicting future trends, it is less appropriate for this specific project as the goal is not to facilitate a common conclusion; rather it is to gather a wide range of the current attitudes and perceptions between pharmacists.

Additionally, since the Delphi method requires expert opinions, the population of focus would shift from general licensed pharmacists to strictly pharmacists with AI based pharmacogenomic experience. Given that AI and its integration into PGx is still relatively new, expecting pharmacists to possess any expert knowledge in this field is unrealistic, making the Delphi method too restrictive [10].

Through the use of Google Forms, an online survey was developed and administered to

licensed pharmacists. Pharmacists were chosen as the target population for this study because their "knowledge and skills of drugs put them at a better position to take the lead and provide clinical services in the area of PGx" [4]. Pharmacists are also healthcare professionals that work directly with drugs and patients at the same time, making their opinions even more essential regarding the issue of PGx and AI. Eligible participants involved all licensed pharmacists, including those without previous exposure to PGx, allowing for a broader range of attitudes and perceptions.

Data Collection Method

Data was collected during a single survey period with each participant completing the survey one time. Data was automatically transferred to a codebook on Google Sheets to keep track of data without collecting personal information. Due to time and networking constraints, this study used a convenience sampling method with voluntary participation. Pharmacists were contacted in-person through pharmacy visits and electronically through emails, which were acquired from publicly accessible sources, mainly university websites where they are employed. The emails written in this process emphasized that participation was completely optional. This process ensures that no confidential or private information was breached, therefore abiding by ethical research guidelines.

Development of Survey

Prior to the creation of the online survey, existing information and research was gathered and synthesized to form a literature review encasing the history of PGx, applications of AI in PGx, and the attitudes of pharmacists towards PGx and AI. The online survey was administered to pharmacists and created through Google Forms as it provides a universally accessible way to create and take online surveys. The survey consisted of 3 main sections: an informed consent form, demographic characteristics, and a short text for the participants to read and provide their perspective on in the following questions.

The survey began with an informed consent form, which provided a brief description of the research study, explaining the purpose of the study and emphasizing that participation was anonymous and voluntary. The study did not offer any form of incentive, only the notion that the data collected from this survey may be used to guide future technological advancements within the pharmaceutical industry. The form required participants to read and agree to the informed consent form. Additionally, participants were required to acknowledge they were licensed pharmacists; this allows only the desired population to proceed into the survey. Should the participants choose not to participate, an option to reject consent was available, and if selected, would exit the participant from the survey. Collectively, all these factors ensure that the study abides by ethical practices.

After choosing to participate, the following section then asked participants for their demographic and professional background in order to contextualize their responses. Participants reported their age, gender, years of practice, and workplace setting. In addition to the general

background information, participants were asked to self-assess their current understanding and level of experience with PGx, if any. These two specific categories were chosen to be the predicted factors that may influence pharmacists' attitudes and perceptions towards the use of AI in PGx. These characteristics provided the necessary context for analyzing any patterns between pharmacists' backgrounds and their perceptions of AI in PGx.

The third section provided pharmacists with an informational overview of what PGx is and various examples of AI usage in PGx. However, only presenting the applications of AI may cause a response bias; in order to combat this, a list of implications that come with using AI in PGx was also given. This text was included to ensure that all participants had some understanding of PGx and AI usage, regardless of previous experience or knowledge. Following this text, Likert-scale and open-ended questions were asked. The Likert-scale questions measured comfortability, likelihood of consulting, willingness to trust, perceived reliability, and concerns, all in regard to AI. Utilizing the Likert-scale allows attitudes and feelings to be converted into quantitative data that can be statistically analyzed [8]. Furthermore, open-ended questions were asked, giving participants the opportunity to elaborate on factors influencing their comfortability, reasons for supporting or not supporting AI results, specific concerns, perceived barriers, and opinions of restrictions or limitations of AI implementation. A thematic analysis was then conducted on the responses given. This data goes beyond the numerical scale, allowing for an in depth analysis.

Data Results

Participant Characteristics

From December 2025 through March 2026, 111 emails were sent to pharmacists and a total of 4 pharmacies were visited in person. However, only 13 pharmacists participated in the survey, resulting in a response rate of 11.71%. The process of recruiting pharmacists online is illustrated in Figure 1. This study utilized Likert-scale items and open-ended questions to examine the attitudes and perceptions of pharmacists.

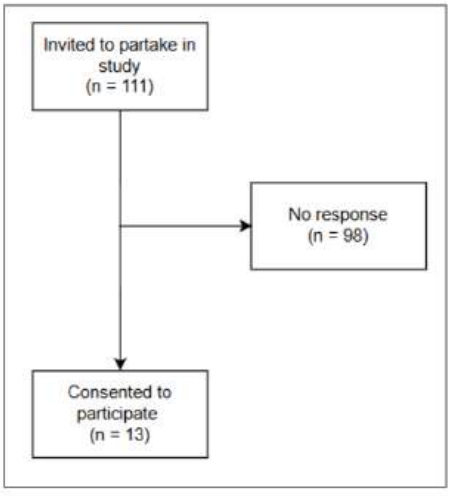


Figure 1: Electronic Email Recruitment Flowchart

Participants were asked to report basic demographic characteristics, including their age, gender, years of practice, workplace setting, and understanding and experience with PGx, shown in Table 1.

Subdemographic	Pharmacists (n=13) Frequency (%)
Age:	
18-25	0 (0%)
26-35	1 (7.7%)
36-45	8 (61.5%)
46-55	2 (15.4%)
55+	2 (15.4%)
Gender:	
Male	8 (61.5%)
Female	5 (38.5%)
Prefer not to say	0 (0%)
Years of Practice	
<1	0 (0%)
1-5	1 (7.7%)
6-10	0 (0%)
11-20	8 (61.5%)
21+	4 (30.8%)
Workplace Setting	
Retail Store	0 (0%)
Hospital/Clinic	5 (38.5%)
Research	4 (30.8%)
Academia	3 (23.1%)
Insurance/ PBM	1 (7.7%)
Understanding of PGx	
1 (I have never heard about PGx)	0 (0%)
2	2 (15.4%)
3	3 (23.1%)
4	0 (0%)
5 (I have an excellent understanding of PGx)	8 (61.5%)
Experience with PGx	
1 (None)	2 (15.4%)
2 (Minimal exposure; read about it but not used)	3 (23.1%)
3 (Some experience; education/training)	6 (46.2%)
4 (Moderate experience; clinical exposure)	2 (15.4%)
5 (Extensive experience; regular use in practice)	0 (0%)

Table 1: Demographic Characteristics

The most frequently reported age group was 36-45 years, consisting of 61.5% of all participants (n=8). The majority of participants were found to be male, as they were 61.5% of all participants as well (n=8). The remaining 38.5% of participants were female (n=5). None of the participants selected “Prefer not to say” for gender.

61.5% of the participants had practiced between 11-20 years (n=8), immediately followed by 30.8% of participants with 21+ years of practice.

In relation to workplace setting, the most common environment was Hospital/Clinic, with 38.5% of participants reporting that option. This was followed by 30.8% of participants reporting Research as a setting (n=4), and 23.1% reporting Academia (n=3).

Participants were also asked to self-report current understanding of PGx; majority reported an excellent understanding (Likert score: 5; n=8; 61.5%). 3 participants expressed a moderate understanding of PGx (Likert score: 3; 23.1%), followed by 2 participants expressing lower levels of understanding (Likert score: 2; 15.4%). No participants selected scores of 1 or 4. Participants were also asked to rank their level of experience with PGx. 46.2% of participants had some experience with PGx (Likert score: 3; n=6); 23.1% of participants had a moderate level of experience (4); 23.1% of participants had some experience (n=3).

Numerical Data

Participants were asked Likert-scale items, with scores ranging from 1 to 5. The results for each item are illustrated in Table 2.

Item	Pharmacists (n=13) Frequency (%)	
1. How comfortable would you feel interpreting AI-supported pharmacogenomic information?		
1 (Strongly Uncomfortable)	3 (23.1%)	
2	1 (7.7%)	
3	2 (15.4%)	
4	4 (30.8%)	
5 (Strongly Comfortable)	3 (23.1%)	
2. AI can improve the accuracy of pharmacogenomic test results.		
1 (Strongly Disagree)	1 (7.7%)	
2	1 (7.7%)	
3	5 (38.5%)	
4	6 (46.2%)	
5 (Strongly Agree)	0 (0%)	
3. AI can benefit clinical decision making in precision medicine.		
1 (Strongly Disagree)	0 (0%)	
2	1 (7.7%)	
3	6 (46.2%)	
4	4 (30.8%)	
5 (Strongly Agree)	2 (15.4%)	
4. The usage of AI introduces ethical and privacy concerns.		
1 (Strongly Disagree)	0 (0%)	
2	4 (30.8%)	
3	1 (7.7%)	
4	5 (38.5%)	
5 (Strongly Agree)	3 (23.1%)	

Table 2: Participant Responses to Likert Scale Items

5. I am willing to use AI as an assistive technology in pharmacogenomic decision making.		
1 (Strongly Disagree)	0 (0%)	
2	2 (15.4%)	
3	3 (23.1%)	
4	5 (38.5%)	
5 (Strongly Agree)	4 (30.8%)	
6. I trust AI-supported pharmacogenomic results to be accurate.		
1 (Strongly Disagree)	2 (15.4%)	
2	3 (23.1%)	
3	6 (46.2%)	
4	2 (15.4%)	
5 (Strongly Agree)	0 (0%)	
7. Overall, I feel positive about the integration of AI in pharmacogenomics.		
1 (Strongly Disagree)	0 (0%)	
2	2 (15.4%)	
3	4 (30.8%)	
4	7 (53.8%)	
5 (Strongly Agree)	0 (0%)	
8. I would need more education and training to feel comfortable using AI in pharmacogenomics.		
1 (Strongly Disagree)	2 (15.4%)	
2	1 (7.7%)	
3	1 (7.7%)	
4	6 (46.2%)	
5 (Strongly Agree)	3 (23.1%)	
9. The pharmaceutical industry would benefit from the widespread use of AI in pharmacogenomics.		
1 (Strongly Disagree)	0 (0%)	
2	1 (7.7%)	
3	6 (46.2%)	
4	5 (38.5%)	
5 (Strongly Agree)	1 (7.7%)	

Table 2, continued: Participant Responses to Likert Scale Items

Item one aimed to measure comfort interpreting AI-supported PGx information. The most reported score was a 4 (n=4, 30.8%), signifying a moderate level of comfort. The second most reported scores were 1 (Strongly Uncomfortable) and 5 (Strongly Comfortable), both of which were reported by 3 participants (n=3, 23.1%). 2 participants chose 3, indicating an average level of comfort (n=2, 15.4%), while only 1 participant chose a score of 2, conveying a lower than average comfort level (n=1, 7.7%)

Item 2 aimed to quantify perception and trust of AI improving accuracy, and the most common response was a 4 (n=6, 46.2%), followed by a score of 3 (n=5, 38.5%). The scores of 1 and 2 were each reported by 1 participant (n=1, 7.7%). No participants chose 5 (Strongly Agree).

Item 3 targeted perceptions of AI benefiting clinical decision making. The most frequent score reported was 3 (n=6, 46.2%), followed by 4 (n=4, 30.8%) and 5 (n=2, 15.4%). 1 participant chose a score of 2 (7.7%) and none chose 1.

Item 4 inquired about the level of concerns associated with AI in PGx. The most common response was a 4 (n=5, 38.5%), indicating that several pharmacists exhibit high levels of concerns surrounding AI. The second highest reported score was a 2 (n=4, 30.8%) and 5 (n=3, 23.1%). 1 participant selected a score of 3 (7.7%), indicating an average level of concern, and none selected 1.

Item 5 aimed to measure willingness to adopt AI as an assistive technology in PGx. The most frequent response recorded was 4 (n=5, 38.5%), followed by 5 (n=4, 30.8%) and 3 (n=3, 23.1%). 2 participants chose a score of 2 (15.4%), and none of the participants chose 1.

Item 6 aimed to measure levels of trust in AI-supported PGx results. The most frequent response recorded was 4 (n=6, 46.2%), followed by 3 (n=3, 23.1%), 4 (n=2, 15.4%), and 1 (n=2, 23.1%). No participants selected a 5, showing no strong agreement with the item.

Item 7 measured overall perceptions of AI integration into PGx. The most frequent response recorded was 4 (n=7, 53.8%), followed by 3 (n=4, 30.8%) and 2 (n=2, 15.4%). No participants selected a 1 or 5, showing neither strong agreement or disagreement with the item.

Item 8 measured perceived need for more training and education. The most frequent response recorded was 4 (n=6, 46.2%), followed by 5 (n=3, 23.1%). 2 participants chose a score of 1 (15.4%), and 1 participant chose a score of 2 (7.7%). 1 participant selected a score of 3 (7.7%).

Item 9 measured perceived benefit of AI to the pharmaceutical industry. The most frequent response recorded was 3 (n=6, 46.2%), followed by 4 (n=5, 38.5%), 1 participant chose 2 (7.7%) and 1 other participant chose 5 (7.7%)%). None of the participants chose 1.

Qualitative Data

Open-ended responses were coded by identifying the main themes and patterns present (see Appendix A). Each question was coded separately, and themes were assigned a specific color. Responses were then highlighted when themes were present using its corresponding color and compiled into frequency tables for analysis.

Data Analysis

Statistical Analysis

This study utilized a non-parametric analytical strategy combined with a thematic analysis to examine the perceptions and attitudes of pharmacists' towards AI as an assistive tool for clinical decision making in PGx. Kendall's Coefficient of Concordance (Kendall's W) was consequently chosen as the method of statistical analysis to measure the level of agreement

among pharmacists as it is a non-parametric test, meaning it does not assume normality and is appropriate for small sample sizes (n=13). Kendall's W produces a W-value ranging from 0-1 through a comparison of ranked items. The W-value is then compared to the Table of Interpretation, which converts the numerical value into an interpretation of agreement, shown in Figure 2.

W	Interpretation
0	No agreement
0.10	Weak agreement
0.30	Moderate agreement
0.60	Strong agreement
1	Perfect agreement

Figure 2: Table of Interpretation for W-value. Figure retrieved from reference [11].

The results gathered in this project showed several ties in the ranks, so the Kendall's W formula seen in Figure 3 was used because it accounts for the previously mentioned ties in the datasets. Figures 4 and 5 show how the S and T variables from Kendall's W were derived.

$$W = \frac{12S}{m^2(n^3 - n) - mT}$$

Figure 3: Kendall's W Formula

$$S = \sum_{i=1}^{n} (R_i - \bar{R})^2$$

Figure 4: Rank Sum Formula (S)

$$T_j = \sum_{i=1}^{g_j} (t_i^3 - t_i)$$

Figure 5: Correction Factor for Tied Ranks (T).
All figures above retrieved from reference [18].

In order to prove statistical significance, the following conditions were used:

- H_0: There is no statistical significance in the level of agreement between participants.
- H_a: There is statistical significance in the level of agreement between participants.

- Reject H_0 : w-value $\geq$ critical-value
- Fail to reject H_0 : w-value < critical-value

Figure 6 shows the critical values used to compare with the W-values.

n\α	0.2	0.1	0.05	0.02	0.01	0.002
4	1.000	1.000	–	–	–	–
5	0.800	0.800	1.000	1.000	–	–
6	0.600	0.733	0.867	0.867	1.000	–
7	0.524	0.619	0.714	0.810	0.905	1.000
8	0.429	0.571	0.643	0.714	0.786	0.857
9	0.389	0.500	0.556	0.667	0.722	0.833
10	0.378	0.467	0.551	0.600	0.644	0.778
11	0.345	0.418	0.491	0.564	0.600	0.709
12	0.303	0.394	0.455	0.545	0.576	0.667
13	0.308	0.359	0.436	0.513	0.564	0.641
14	0.275	0.363	0.407	0.473	0.516	0.604
15	0.276	0.333	0.390	0.467	0.505	0.581
16	0.250	0.317	0.383	0.433	0.483	0.567
17	0.250	0.309	0.368	0.426	0.471	0.544

Figure 6: Critical Values. Figure retrieved from reference [19].

Kendall's Tau-b (τb), seen in Figure 7, was used to examine associations between ordinal data and outcome variables.

$$\tau_B = \frac{n_c - n_d}{\sqrt{(n_0 - n_1)(n_0 - n_2)}}$$

Figure 7: Kendall's Tau-b (τb) Formula. Figure retrieved from reference [15].

Kendall's W was conducted on two constructs: "Perceptions of AI" and "Attitudes towards AI." To create each construct, three Likert-scale survey items were chosen that best represented the construct. Then the mean score for each item in the construct was calculated, and a composite mean ($\bar{x}$) was determined to illustrate the general trends. The following conditions were used to interpret the composite means:

- Generally positive: $\bar{x}>3$
- Neutral: $\bar{x}=3$
- Generally Negative: $\bar{x}<3$

These descriptive statistics were used alongside the W-values to support overall perception and attitude patterns.

The "Perceptions of AI" construct was created through three items, $Q1_p$, $Q2_p$, $Q3_p$, respectively: "AI can improve the accuracy of pharmacogenomic test results", "AI can benefit clinical decision making in precision medicine", and "The pharmaceutical industry would benefit

from the widespread use of AI in PGx." These Likert-scale statements were chosen because they actively gather how the pharmacists perceive AI in PGx. Participants were asked to choose between 1 (Strongly Disagree) through 5 (Strongly Agree) for all items in this construct. Table 3 organizes the ratings of the pharmacists for each item in the construct, as well as the numerical values that were used to find the W-value.

	n=3							
Participants (m)	Q1p Response	Q2p Response	Q3p Response	Rank Q1p	Rank Q2p	Rank Q3p	Rank Sum (Ri)	(Ri - R)^2
1	3	3	3	5	4.5	4.5	14	49
2	3	3	4	5	4.5	10	19.5	2.25
3	2	5	4	2	12.5	10	24.5	12.25
4	4	5	3	10.5	12.5	4.5	27.5	42.25
5	3	3	3	5	4.5	4.5	14	49
6	4	2	3	10.5	1	4.5	16	25
7	1	3	3	1	4.5	4.5	10	121
8	3	3	3	5	4.5	4.5	14	49
9	4	4	5	10.5	9.5	13	33	144
10	4	4	4	10.5	9.5	10	30	81
11	4	4	4	10.5	9.5	10	30	81
12	3	3	2	5	4.5	1	10.5	110.25
13	4	4	4	10.5	9.5	10	30	81
							Mean Rank (R)	S
							21	847

Value	Q1p Count	Q1p (t^3-t)	Q2 Countp	Q2p (t^3-t)	Q3p Count	Q3p (t^3-t)		W-value:
1	1	0	0	0	0	0		0.6032763533
2	1	0	1	0	1	0		
3	5	120	6	210	6	210		
4	6	210	4	60	5	120		
5	0	0	2	6	1	0		
		330		276		330		
					T=	936		

Table 3: Kendall's W for Perceptions of AI Construct

The calculated W-value is ~0.60; the Table of Interpretation signifies a Strong Agreement. The Critical Value was 0.436, given that n=13 at a significance level of 0.05. Therefore, the null hypothesis is rejected and the findings are statistically significant because the W-value (0.60) ≥ Critical Value (0.436). $\bar{x}$ was calculated to be 3.41, and since 3.41>3, the analysis demonstrates that there is a statistically significant level of agreement among pharmacists, indicating generally positive perceptions.

The "Attitudes towards AI" construct was also created with three Likert-scale items, $Q1_a$, $Q2_a$, $Q3_a$, respectively: "I am willing to use AI as an assistive technology in pharmacogenomic decision making", "I trust AI-supported pharmacogenomic results to be accurate.", and "Overall, I feel positive about the integration of AI in PGx." These statements were chosen because they best measure how the pharmacists feel towards AI in PGx. Participants were asked to choose between 1 (Strongly Disagree) through 5 (Strongly Agree) for all items in this construct. Table 4 organizes the ratings of the pharmacists for each item in the construct, as well as the numerical values that were used to find the W-value.

	n=3							
Participants (m)	Q1a	Q2a	Q3a	Rank Q1a	Rank Q2a	Rank Q3a	Rank Sum (Ri)	(Ri - R)^2
1	3	2	3	3.5	4	4.5	12	81
2	5	3	3	11.5	8.5	4.5	24.5	12.25
3	5	3	4	11.5	8.5	10	30	81
4	5	4	4	11.5	12.5	10	34	169
5	2	2	2	1.5	4	1.5	7	196
6	5	2	4	11.5	4	10	25.5	20.25
7	3	1	3	3.5	1.5	4.5	9.5	132.25
8	2	1	2	1.5	1.5	1.5	4.5	272.25
9	4	3	4	7	8.5	10	25.5	20.25
10	4	3	4	7	8.5	10	25.5	20.25
11	4	4	4	7	12.5	10	29.5	72.25
12	4	3	3	7	8.5	4.5	20	1
13	4	3	4	7	8.5	10	25.5	20.25
							Mean Rank (R)	S
							21	1098

Value	Q1a Count	Q1a (t^3-t)	Q2a Count	Q2a (t^3-t)	Q3a Count	Q3a (t^3-t)		W-value:
1	0	0	2	6	0	0		0.768907563
2	2	6	3	24	2	6		
3	2	6	6	210	4	60		
4	5	120	2	6	7	336		
5	4	60	0	0	0	0		
		192		246		402		
					T=	840		

Table 4: Kendall's W - Attitudes Towards AI Construct

The calculated W-value is ~0.77; the Table of Interpretation signifies a Strong to Perfect Agreement. The Critical Value was 0.436, given that n=13 at a significance level of 0.05. Therefore, the null hypothesis is rejected and the findings are statistically significant because the W-value (0.77) ≥ Critical Value (0.436). $\bar{x}$ was calculated to be 3.28, and since 3.28>3, the analysis demonstrates that there is a statistically significant level of agreement among pharmacists, indicating generally positive attitudes.

Kendall's Tau-b (τb) was used to find correlations between "PGx Understanding" and "PGx Experience" and outcome variables, which were Comfort, Trust, Ethical Concerns, and Need for Training, all in relation to AI. The Table of Interpretation for Kendall's Tau-b is shown in Figure 8 and was used to interpret the τb value. The p-value in the table was interpreted at a level of significance at 0.05. Due to the small sample size (n=13), emphasis was placed more on strength and direction of correlations instead of statistical significance, as small sample sizes reduce reliability of p-values.

Size of Correlation	Interpretation
0.90 to 1.0 (−0.90 to −1.0)	Very high positive (negative) correlation
0.70 to 0.90 (−0.70 to −0.90)	High positive (negative) correlation
0.50 to 0.70 (−0.50 to −0.70)	Moderate positive (negative) correlation
0.30 to 0.50 (−0.30 to −0.50)	Low positive (negative) correlation
0.00 to 0.30 (−0.00 to −0.30)	Negligible correlation

Figure 8: Table of Interpretation for τb. Figure retrieved from reference [2].

The table below shows each pharmacists' ranking of their understanding of PGx and their corresponding responses to each of the outcome variables. Rankings for understanding were from 1 (I have never heard about PGx) to 5 (I have an excellent understanding of PGx).

Participants	PGx Understanding	Comfort Interpreting	Trust	Ethical Concerns	Need for Training
1	3	3	2	4	5
2	5	4	3	4	2
3	5	4	3	2	4
4	5	5	4	2	4
5	2	1	2	4	5
6	5	3	2	2	1
7	5	5	1	2	3
8	5	1	1	5	4
9	2	1	3	5	5
10	5	5	3	4	1
11	5	4	4	3	4
12	3	2	3	5	4
13	3	4	3	4	4

Table 5: Understanding of PGx and corresponding responses to outcome variables for each participant

Using the values in the table above, the following table was created:

Understanding		
Outcome	**Kendall's τb**	**Correlation Interpretation**
Comfort	0.585	Moderate positive
Trust	0.077	Neglible
Ethical Concerns	-0.518	Moderate negative
Need for Training	-0.691	Moderate negative

Table 6: τb values for understanding and outcome variables with correlation interpretations

According to the table above, a moderate positive correlation was found between understanding of PGx and Comfort (τb=0.585). A moderate negative relationship was found between understanding of PGx and Ethical Concerns and Need for Training, τb=-0.518 and τb=-0.691, respectively. However, a negligible correlation was found between understanding of PGx and Trust.

The table below shows each pharmacists' ranking of their experience with PGx and their corresponding responses to each of the outcome variables. Rankings for experience were from 1 (None) to 5 Extensive experience (Regular Use in Practice).

Participants	PGx Experience	Comfort Interpreting	Trust	Ethical Concerns	Need for Training
1	3	3	2	4	5
2	5	4	3	4	2
3	5	4	3	2	4
4	4	5	4	2	4
5	2	1	2	4	5
6	5	3	2	2	1
7	5	5	1	2	3
8	5	1	1	5	4
9	2	1	3	5	5
10	5	5	3	4	1
11	4	4	4	3	4
12	2	2	3	5	4
13	4	4	3	4	4

Table 7: Experience with PGx and corresponding responses to outcome variables for each participant

Using the values in the table above, the following table was created:

Experience		
Outcome	**Kendall's τb**	**Interpretation**
Comfort	0.46	Low positive
Trust	-0.191	Neglible
Ethical Concerns	-0.431	Low negative
Need for Training	-0.724	High negative

Table 8: τb values for experience and outcome variables with correlation interpretations

According to Figure 8, there is a low positive correlation between Experience with PGx and Comfortability using AI (τb=0.46). Additionally, there is a low negative correlation between experience with PGx and Ethical concerns (τb=-0.431). There is a high negative correlation between experience with PGx and Need for Training (τb=-0.724). However, there is a negligible correlation between experience with PGx and Trust in AI (τb=-0.191).

Thematic Analysis

To analyze the qualitative responses gathered, a thematic analysis was performed by coding the responses into the main themes found in each question; from there, interpretations were made based on the main themes found and their frequency.

In order to gather contextual information about pharmacists' previous experience with PGx, an open-ended question was asked to the participants to gauge levels of experience.

What types of AI-supported pharmacogenomic tools, if any, have you used in practice, and what was your opinion of them?		
Theme	**Count**	**Percentage**
No AI tools used	12	75%
Algorithms used instead of AI	1	6.25%
Institutional Interest in Adopting AI	2	1.25%
AI used for literature/summaries/planning	1	6.25%

Table 9: Current Use of AI-supported Tools in Practice

As shown in Table 9, twelve participants indicated that they had not used AI-supported pharmacogenomic tools, illustrating that AI integration into PGx is still not well developed. Although the majority of participants do not have experience with AI tools, one participant reported that they use AI for scientific literature summaries and planning, suggesting a more administrative use of AI. One participant also reported using algorithms instead of AI, reflecting the conventional tools used currently. A smaller portion expressed interest in adopting AI for pharmacogenomic use, highlighting that there may be potential implementation in the future. Overall, these findings suggest that AI in PGx is still at an early stage; pharmacists are aware of its potential but still have not implemented it.

Table 10 illustrates the main themes found in responses to the open-ended question that aimed to examine whether participants would or would not provide a recommendation based on AI-supported pharmacogenomic test results.

Please explain why you would or would not provide a recommendation of medicine based on AI-supported pharmacogenomic test results.		
Theme	**Count**	**Percentage**
Limited clinical and research evidence	4	33.33%
AI should only assist; professionals should verify	6	50.00%
Lack of guidelines/expertise	2	1.67%
Time-saving	2	1.67%

Table 10: Factors Influencing Willingness to Provide AI-based Recommendations

Six participants reported that they believe that AI should only be used as guidance and that professionals should still verify information, highlighting themes of slight acceptance but still caution. This reinforces the idea of AI being strictly a support tool. Four participants noted that limited clinical and research evidence reduce their favorability of providing such recommendations; this shows how AI implementation is restricted and therefore shapes pharmacists' feelings towards AI adoption. Smaller groups indicated that time saving could be a benefit but the lack of guidelines and expertise surrounding the topic make it difficult to provide such recommendations. Altogether, the findings from this question highlight that pharmacists are not completely against AI, but are willing to integrate it into decision making with some restrictions.

Table 11 shows the key themes found in responses to the question about possible interventions that would make pharmacists more comfortable with AI.

If you felt the need for more education and training, what type of support would make you feel more comfortable?		
Theme	**Count**	**Percentage**
Yes	12	40.00%
No	1	3.33%
More research evidence	3	10.00%
Understanding AI training sources	6	20.00%
Expert consultations	3	10.00%
Continuing Education	3	10.00%
Certificate Programs	2	6.67%

Table 11: Types of Support in Education and Training

Twelve participants felt the need for more education and training, suggesting that hesitancy to adopt AI may come from lack of familiarity with the technology rather than just inherent resistance. Pharmacists may be more willing to use AI once they achieve a better understanding and grasp of it. Understanding AI aligns with the second most frequent theme (six participants) found: Understanding AI training sources. This suggests that pharmacists are concerned about the reliability of the technology. Other responses include expert consultations, continuing education, and certification. All of these responses show how pharmacists would need to see some form of training or intervention in order to feel more comfortable.

Table 11 shows the variety of concerns reported by pharmacists about the usage of AI in PGx.

Are there any specific concerns do you have about AI in pharmacogenomics (e.g., accuracy, patient privacy, professional responsibility)?		
Theme	**Count**	**Percentage**
Guidline Issues	3	1.25%
Privacy	7	29.17%
Reliability	1	4.17%
Professional Responsibility	3	1.67%
Accuracy	9	37.50%
N/A	1	4.17%

Table 11: Concerns about AI in PGx

Nine participants expressed concerns about accuracy; seven participants expressed privacy concerns. Smaller groups of participants expressed concerns such as guideline issues, professional responsibility, and reliability. One participant had no specific concerns about the topic. These findings show that participants strongly believe that accuracy and privacy are the main concerns.

Table 12 shows the main barriers identified in participants' responses when asked what barriers prevent AI-tools from being implemented in PGx.

In your opinion, what are some barriers, if any, that prevent pharmacists from using AI supported pharmacogenomic tools?		
Theme	**Count**	**Percentage**
Limited Research	1	6.67%
Lack of training in field	7	46.67%
Legal Issues	2	13.33%
Complexity of Data	2	13.33%
Fear of AI	2	13.33%
N/A	1	6.67%

Table 12: Barriers preventing AI adoption for pharmacists

The biggest factor was a limited amount of research, with seven participants expressing that. Legal issues and complexity of data were both reported by two participants. One participant reported limited research as a barrier while one participant expressed no barriers. Overall, these results show how educational and systematic issues are the main barriers that prevent pharmacists from using AI supported pharmacogenomic tools.

Discussion

This study aimed to examine the attitudes and perceptions of pharmacists towards the usage of AI as an assistive tool for clinical decision making in PGx. The findings from this study indicate that pharmacists show a cautious optimism towards the adoption of AI. Performing a Kendall's W statistical test resulted in W-values of 0.60 and 0.77 for perceptions and attitudes towards AI, respectively. Since both values exceeded the critical value of 0.436, statistical significance was demonstrated, as well as a strong level of agreement for both factors. Additionally, the both constructs yielded composite means over 3, suggesting that pharmacists show generally positive perceptions and attitudes towards AI in pharmacogenomics. However, this optimism is restricted because of the concerns expressed by the pharmacists. According to Table 11, nine out of the thirteen participants reported accuracy as a major concern, and seven participants expressed additional privacy concerns. Pharmacists show optimism but a high percentage still express concerns, suggesting that pharmacists are not inherently opposed to the technology, but are instead opposed to using it unconditionally. When asked what types of support would make them feel more comfortable, participants most frequently reported that understanding the AI training sources would be most beneficial, followed by more evidence supported by research and expert consultations. It is reasonable for pharmacists to have high standards for such technologies, given that errors in PGx decision making can be fatal due to adverse reactions [16].

Kendall's Tau-b statistical test revealed that pharmacists with greater understanding of PGx report higher levels of comfort interpreting AI-supported PGx information ($\tau b=0.585$) and a lower perceived need for further training or education ($\tau b=-0.691$). This aligns with the results in Table 11, where twelve out of the thirteen pharmacists listed needing more training/experience to feel more comfortable. Surprisingly, a negligible correlation was found between trust in AI in both understanding and experience of PGx. This implies that trust may be based on external factors rather than understanding and experience.

Overall, these findings support the initial hypothesis and suggest that pharmacists are open to the adoption of AI into PGx clinical practice, but are held back due to limited awareness of the field and no sufficient research to back up effectiveness. In order to implement AI across a wide range, more education, training, and awareness are needed, as well as stronger evidence and development of uniform and standardized guidelines.

Limitations

There were several limitations to this study. The sample size of 13 pharmacists is insufficient to be representative of the pharmacist population, greatly limiting the generalizability and increasing the probability of sampling bias. The low response rate of 11.7% only amplifies this issue; since participation was voluntary, voluntary response bias may have influenced the results as pharmacists who chose to participate may have more interest in this field, and therefore, different attitudes and perceptions towards AI than those who did not participate. Additionally, the accuracy of the responses cannot be verified because this study depends on anonymity and self-reported data. Self-reported data may introduce bias and

therefore create inaccuracies within my data. The informational overview in my survey may have influenced participant responses, although both positive and negative features were presented.

Future Directions & Conclusion

Future research on this topic should focus on expanding sample size to improve generalizability and provide a more complete understanding of pharmacists' attitudes and perceptions of AI in PGx. More factors should be considered outside of Understanding and Experience of PGx to improve diversity of the sample, such as geographic location or pharmacy speciality. Future research could potentially introduce other healthcare professions as a demographic, where results could be compared across the different professions. Overall, this study demonstrates that pharmacists exhibit positive attitudes and perceptions towards the usage of AI in PGx, but also express concerns, including privacy and accuracy. These concerns can be addressed through further development of AI and guidelines. While the small sample size of 13 limited generalizability and representation, this study addressed a significant gap by investigating AI and PGx in relation to pharmacists. As the field of AI and PGx continue to develop rapidly, it will be increasingly important to address pharmacists' concerns when implementing AI into pharmacogenomic practice.

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Appendix A

Thematic coding sheets of qualitative responses for each open-ended question.

Theme	Color
No AI tools used	
Algorithms used instead of AI	
Institutional Interest in Adopting AI	
AI used for literature summaries/planning	

What types of AI-supported pharmacogenomic tools, if any, have you used in practice, and what was your opinion of them?
None
Non- I use my own judgement based on experience and research
Currently none, our institution is looking into ways we can use AI to identify patients who would benefit most from testing. We are also looking into ways to help give patients pre-pharmacogenetic test education with AI; this would help better set expectations & decrease our workload. We're in the beginning planning/talking phases but I'm very optimistic it will be beneficial to us and patients!
I have not used a AI supported PGx tool in practice
N/A
I have not used AI PGx tools; all clinical computer decision support that we use relies on simple algorithms.
I haven't used in practice, but I've seen a demo of an app a friend created and it looks very helpful.
none
None
I use AI tools like the University of Michigan's (UM) private versions of Gemini and GPT mostly to help with writing. Sometimes I use Perplexity AI to do quick literature searches and summaries for scientific questions. None of these are specifically for pharmacogenomics, except I often write scientific manuscripts on pharmacogenomics. We also use National Language Processing to collect research data from clinician's notes in the UM electronic health records for PGx studies. We are also teaching students to use AI for summarizing PGx topics for a lay audience, design PGx research studies, understand the field, and assist with patient cases and treatment plans.
None
n/a
AI is not really used for pharmacodynamics in my practice. We rely more on labs and blood work

Theme	Color
Limited clinical and research evidence	
AI should only assist; professionals should verify	
Lack of guidelines/expertise	
Time-saving	

Please explain why you would or would not provide a recommendation of medicine based on AI-supported pharmacogenomic test results.
Currently, the number of medications in which we have robust evidence to say that pharmacogenomic guidance is truly impactful is pretty limited. While this will likely continue to grow in the future, based on currently available research, impactful pharmacogenomic-based dosing recommendations are limited to a relatively small subset of drugs.
I would use the AI data as presented but often some data have not been published and one needs to also take into consideration other issues that the patient has especially in respect of phenoconversion
I would just need more information to be confident in its interpretation of results.
lack of clear guidelines with some genes
Lack expertise in this area
I have significant PGx experience, so I know enough to be suspicious of incorrect AI PGx recommendations. Even still, I would likely confirm the provided information is correct before making a recommendation.
Depends on evidence/resources AI is using to provide interpretation/recommendation
I would only after personally checking the recommendation.
Interpretation should still be done by professional with an understanding of the field
There is a lot of pharmacogenetic studies and resources available now, and clinicians are busy, so AI could be a tool to help quickly and efficiently interpret patient information and make treatment recommendations. However, I also have the training and experience necessary as both a pharmacist and a PGx expert in order to verify the accuracy and completeness of the AI results.
I would provide recommendations if the platform shared and used quality references. I think I would be open to it because it will be a quick way to apply these things to patient care.
Recommendations should be done by experts in the field, not only the AI. Even after the AI recommendation, the information should be checked for accuracy.
I would be nervous to rely on AI fully. I rather use AI to help but then decide myself if I should proceed with the advice

Theme	Color
More research evidence	
Understanding AI training sources	
Expert Consultation	
Continuing education	
Certificate programs	

If you felt the need for more education and training, what type of support would make you feel more comfortable?
Robust research to demonstrate its utility to improve clinical outcomes
Postgraduate education, peer experts, case studies, public health experts
I'd want a good understanding of the information used to teach the model.
High level seminar(s), professional certificate training programs
Completion of a certificate program
I would like to know what sources the AI model uses.
understanding literature/resources AI is pulling to make a recommendation; prompts
how the AI model is built and learns, what inputs go into the AI model
Continuing education programs or formal courses
n/a
Webinar, computer training.
More studies to prove effectiveness, what sources AI is trained on
Chat room with clinical experts

Theme	Color
Guideline Issues	
Privacy	
Reliability	
Professional Responsibility	
Accuracy	

Are there any specific concerns do you have about AI in pharmacogenomics (e.g., accuracy, patient privacy, professional responsibility)?
all of the above in this question
Ai can provide some "advice" but unless it is based on national/international guidelines then it is prone to overemphasis of the benefits of pharmacogenomics- also one has to question what publications Ai has synthesised the data and whether it is actually correct
Overgeneralization, missing key components it is not "trained" to look for (e.g., applying phenconversion accurately). I would be considered about professional responsibility although I think that could be resolved with extra training.
Regulatory issues, cost, data privacy
Reliability
Clinicians receiving incorrect recommendations and not realizing they are flawed.
accuracy
patient privacy
Accuracy and privacy are my main concerns
accuracy and patient privacy are still concerns
NA
Privacy concerns, accuracy, uniform regulations across field
Pretty much accuracy, privacy, relying on it too much

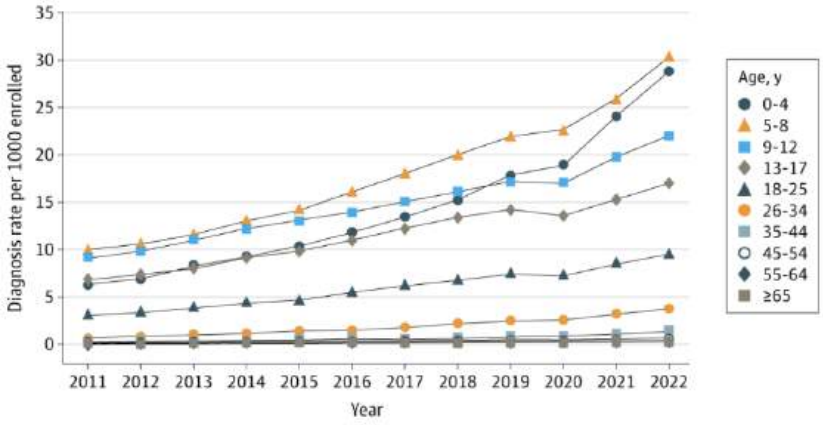
Exploring the Correlation between Verbal Working Memory Capacity and Conversational Contingency patterns in Children with Autism Spectrum Disorder (ages 6-12)

By Angel Ajish Yohannan

Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by differences in social communication and restricted or repetitive behaviors. Pragmatic language difficulties including challenges with reciprocal conversation and interpreting social cues, are common among autistic children (National Institute of Deafness and Other Communication Disorders, 2020). It has become increasingly recognized in recent years, where in 2022, the Centers for Disease Control and Prevention (CDC) recorded that ASD prevalence in children aged 8 years was found to be 32.2 per 1,000 children (Shaw, 2025). A cross-sectional study by Grosvenor (2024) found autism diagnoses increasing most rapidly among children ages 5–8 (Figure 1). These ages fall under the typical school-age children group (6-12), a critical period for the development of conversational skills and pragmatic language use. However, these functions are often impaired in children with ASD, particularly pragmatic language capabilities.

Figure 1: Annual Autism Diagnosis Rates Among Members at Mental Health Research Network Sites From 2011 to 2022, Stratified by Age Group (Grosvenor 2024)



This issue is particularly significant for school-aged children with ASD, as impairments in communication and pragmatic language may interfere with peer relationship development and social acceptance. Pragmatic language includes being able to understand and use nonverbal communication cues, maintain conversational turns, and respond appropriately to social context (Mohan, 2025). Compared to neurotypical children, where pragmatic language typically develops through everyday social interaction, children with ASD often experience a substantially different developmental pattern in pragmatic language that inhibit their ability to participate in contextually appropriate conversational turn-taking, otherwise known as conversational contingency (Mohan, 2025; Paul, 2008).

A possible factor that may contribute to variability in conversational contingency is verbal working memory, the cognitive system responsible for the temporary storage and active usage of auditory and language-based information. Verbal working memory allows a speaker to process a partner's preceding utterance while simultaneously creating a contextually relevant

response. Limitations in this memory may therefore affect turn-taking relevance (contingency). Despite growing research on pragmatic language deficits in ASD, limited studies have directly examined whether verbal working memory capacity is associated with variability in conversational contingency. Therefore, examining the potential overlap between verbal working memory deficits and conversational contingency challenges in children with ASD is critical to clarifying whether cognitive processing capacity influences conversational relevance.

Pragmatic Language Deficits in ASD

Pragmatic language difficulties are well documented in autistic populations, as "pragmatic impairment has become a critical aspect of language development in autistic children" (Liu, 2024). To confirm this, Paul et al. (2008) found significant impairments in approximately one-third of pragmatic language behaviors among autistic adolescents, particularly in topic management, information management, and reciprocity. While this study established pragmatic language impairments in ASD, it does not explore cognitive mechanisms related to these difficulties, such as working memory.

Conversational Contingency in Childhood

Conversational turn-taking refers to the alternating exchanges between speakers that maintain the flow and mutual understanding of a conversation (Nordquist, 2025). Conversational contingency specifically refers to producing responses that are directly relevant to a previous speaker's statement. It is measured by contingent turns, which are responses that are directly related to a preceding speaker's statement, demonstrating attention and understanding to what was previously said. A study conducted by Pagmar et al. (2022) investigated predictors of children's conversational contingency in a longitudinal study of 40 typically developing Swedish-speaking five-year-olds. Using semi-structured conversations with standardized prompts, children's responses were coded as contingent, non-contingent, minimal, or missing (Figure 2). The authors found that children with larger receptive vocabularies were more likely to participate in conversations at all, as indicated by fewer missing turns. However, having a larger vocabulary did not make children more likely to produce responses that were relevant to the previous speaker's statement (contingent turns). This distinction suggests that structural language ability alone cannot account for conversational success, as children may still struggle to process a conversation and respond, raising the possibility that other cognitive resources, particularly those involved in temporarily storing and manipulating verbal information, may influence whether a child can produce a contextually contingent response.

The authors note that this pattern was also found in verbally fluent children with autism. While they may demonstrate age-appropriate vocabulary and grammar, they still continue to experience difficulty maintaining reciprocal, relevant conversation. This suggests language skills alone do not explain differences that constrain autistic children's ability to process content and produce contingent responses in real-time conversation, which motivates the study's focus on

examining potential cognitive mechanisms such as verbal working memory, that may be related to these difficulties.

Cognitive Contributors to Conversational Topic Maintenance

Difficulties in pragmatic language and conversational contingency observed in children with autism are often linked to broader cognitive processes that support communication, such as executive functioning, a set of higher-order cognitive skills responsible for planning, attention, cognitive flexibility, and working memory (Diamond, 2013). Impairments in executive functioning are widely recognized as a core feature of ASD (Duncan, 2022). Research comparing children with ASD to their neurotypical peers consistently demonstrates underperformance on executive functioning tasks, particularly those assessing working memory capacity. A recent study by Abbot-Smith et al. (2024) consisted of three pre-registered studies comparing conversational topic maintenance in autistic and neurotypical children aged 5–9 years. Although autistic children were more likely to produce off-topic or minimal verbal responses, they still provided topic-supporting responses in 62% of conversational turns, suggesting that conversational ability exists on a continuum rather than as a uniform deficit. However, the absence of strong cognitive predictors may reflect the broad measurement of executive functioning, suggesting that specific processes such as working memory necessitate closer investigation. The study found theory of mind ability significantly predicted topic-supporting responses in both groups, but did not explain unique variance in contingent responding. Producing a truly contingent response requires the speaker to recall the previous utterance while forming a reply, a process that may place additional demands on working memory.

These findings may not fully capture the conversations children encounter in everyday social settings. These findings highlight the need to examine additional cognitive factors such as verbal working memory as a possible predictor to understand whether verbal working memory correlates with individual differences in contingent responding, especially in children with ASD who might otherwise appear verbally competent.

Verbal Working Memory in ASD

Working memory temporarily stores and manipulates information necessary for reasoning and language processing. The Baddeley and Hitch model identifies the phonological loop as the subsystem responsible for temporarily storing and rehearsing auditory information, making it particularly relevant to verbal working memory and conversation (Baddeley, 2003). Working memory impairments in individuals with ASD appear to persist regardless of age or any concurrent developmental conditions, suggesting a stable cognitive vulnerability rather than a developmental delay (Duncan, 2022). Similarly, as part of executive functioning, verbal working memory capacity deficits are also evident in children diagnosed with ASD. Longitudinal data recorded in a study in the academic journal Autism indicated that autistic children enter formal schooling with significantly lower verbal working memory capacity with disparities evident as

early as kindergarten and persisting across elementary school (Kim, 2023). These findings suggest that verbal working memory, powered by the phonological loop, represents a stable area of difficulty in ASD, which could help explain why some children struggle to produce contextually relevant conversational turns despite having age-appropriate vocabulary and grammar.

Additionally, studies consistently demonstrate that children with ASD show significantly poorer performance on verbal working memory tasks, such as Digit Span, with large effect sizes observed across age groups, which further suggests a persistent executive functioning impairment (Chen, 2016). Although these studies establish the existence of these deficits, they do not directly investigate whether these impairments correlate with challenges in conversational contingency.

Verbal Working Memory and Social Communication

While the relationship between conversation and cognition has been studied in children, fewer studies have directly examined the role of working memory in everyday social interaction. Ishiguro et al. (2024) addressed this gap by investigating the association between working memory capacity and face-to-face social interaction in two adult samples. Using a listening span task (LSPAN) to measure working memory and multiple social network questionnaires, the authors found that working memory recall was positively correlated with social network size, including face-to-face interactions, even after accounting for online communication (Ishiguro et al., 2024). Although this research examined adults rather than children, the findings suggest that working memory may help individuals maintain conversational context. Whether similar factors influence contingent responding in autistic children remains largely unexplored.

For the current study, this research highlights a potential cognitive mechanism that could explain why some children with ASD struggle with maintaining contextually appropriate conversational turns, even when their vocabulary and grammar are age-appropriate. Integrating this perspective helps portray verbal working memory as not only a cognitive skill but also a facilitator of live social communication. This research is relevant because it offers a cognitive basis for understanding how working memory may support sustained social engagement, a mechanism that has not yet been adequately explored in autistic children and is central to the focus of the present exploratory research.

Gaps in the Research

Although conversational contingency deficits in children with ASD are well documented, existing research has not sufficiently explored the cognitive mechanisms that could be enforcing these difficulties in conversation. Particularly, no study has directly investigated whether limitations in verbal working memory capacity could be linked to reduced contingent responding during social exchanges. Most prior work has focused either on language abilities or broad cognitive abilities such as theory of mind, leaving the possible role of verbal working memory in conversation unaddressed. Therefore, there is limited evidence and research

explaining how such working memory constraints may impact autistic children's ability to maintain contingent turns. To fill the gap and to connect the previous research, this study aims to provide a more comprehensive understanding of verbal working memory capacity and whether its patterns correlate with variability in conversational contingency in children with ASD.

Methodology

This study uses a mixed-methods correlational design measuring both variables, verbal working memory capacity and conversational contingency, in children with ASD. Quantitative data are collected through a modified Listening Span (LSPAN) task to assess verbal working memory capacity and a structured conversational contingency task to measure relevant turn-taking. Qualitative observations are recorded during both tasks, noting patterns in attention, distractibility, and other behavioral patterns. These observations contextualize the quantitative scores, helping explain individual differences in performance and guiding potential interventions.

A correlational approach would be the most efficient and ethical approach to identify a relationship between verbal working memory and conversational contingency, as it allows the study to explore whether these two skills share a positive or negative correlation without changing a participant's environment or behavior. This is significant because for children with ASD, "adapting to new environments or situations can be an especially difficult task, leading to heightened anxiety and resistance to change" (HANDS Center for Autism 2024). To account for a smaller sample size, many research studies utilize a mixed-methods study that employs strong qualitative data (Hennink, 2022). Therefore, this mixed-methods design effectively measures verbal working memory and conversational contingency performances numerically while also comparing patterns of attention, engagement, and social behavior, offering a holistic representation of each participant's cognitive and pragmatic profile.

This study utilized methods that measured verbal working memory and conversational contingency separately. Participants first completed a modified version of the Listening Span Task (LSPAN) to assess verbal working memory. The LSPAN is a complex span task, requiring participants to store information while performing a processing task, which in this case is judging whether sentences were true or false, and then recalling the final word of each sentence (Mathy, 2018; Bazan, 2021). This type of assessment ensures evaluation of working memory under cognitive load rather than simple recall. However, the traditional LSPAN structure is not developmentally appropriate for children with ASD, as it places high simultaneous demands on language processing, inhibitory control, and serial recall (Bazan, 2021). Accordingly, the LSPAN was adjusted to use recall following the true/false question of each sentence rather than after three sentences. This modification reduced task complexity while preserving the dual-processing demands of verbal working memory assessment.

The modified LSPAN included 35 different statements across seven difficulty levels (2, 3, 4, 5, 6, 7, & 8-word sentences) (Appendix A). Participants heard each sentence out loud (e.g., "Dogs can quack"), judged it as true or false, and recalled the final word. Each word level included three sentences plus two backups, which were only used to ensure a participant couldn't

move past a difficulty level. The task was stopped when a participant failed to recall fewer than two words correctly at a level, with the highest successful word level (2 or more words correct) recorded as the participant's Highest Functional Span (HFS). Each correct final word was scored as 1 point, with incorrect or omitted words scored as 0. The total score, summed across all sentences divided by total sentences attempted, indicated verbal working memory capacity (LSPAN score), with higher scores reflecting greater ability to retain and process spoken information under cognitive load.

Participants then engaged in a 3-minute naturalistic conversation with neutral prompts (e.g., "Tell me about something you like doing") (Appendix B). Responses were coded as a conversational turn using an established scheme (Figure 2), classified as contingent, non-contingent, minimal, non-verbal, or null. Conversational contingency was calculated as the proportion of contingent responses out of the total number of responses produced during the conversation, with higher values reflecting greater contingency.

Figure 2: Conversational Coding Scheme (Abbot-Smith 2024)

Example responses were to the prompt "I have a pet"

	Coding category	Definition	Example response from Study 1 data
Sentential response	Contingent Responses	Multi-word utterances which are relevant and which elaborate on the content of the conversation partner's turn (Bloom et al., 1976)	P12: 'I've got 2 pets' P18: 'Is it a tiger?'
	Non-contingent Responses	Either (i) were not on the topic of (or only very tangentially related to) the immediately preceding turn, ii) had a very unclear meaning or iii) were 'bizarre' or iv) switching to talk about something in the environment (Nadig et al., 2010)	P51: 'Yeah there's a new boy and he punched him like some chocolate and he went [NOISE] in the face
Minimal response	Specific minimal responses	Were either (i) one word utterances which were clearly specific to the content of the preceding turn (e.g. E: 'I have a pet' C: '(A) Doggy?') or ii) short phrases which encouraged the conversation partner to keep talking (e.g. 'Did you?', 'Was it?') but which did not elaborate on the topic (Bavelas et al., 2000)	P15: A doggy! P31: What? P28: Have you? (Other possible responses: 'Really?', 'Cool!')
	Generic minimal responses	Were single words or short phrases which might be acknowledgements of the conversation partner's turn but which could potentially be uttered without the speaker listening to or processing the conversation partner's turn (Bavelas et al., 2000; Jefferson, 1984)	P13: 'Mm-hmm' (Other possible responses: 'Yeah' 'Oh' 'Uh-huh' 'Right')
No language used	Non-verbal Responses	Non-verbal behaviour which acknowledge or respond to the conversation partner's turn	Nods Raises eyebrows Smiles in response
	Null response	The child either looked at something in the room or else looked at the experimenter's face with no discernible reaction	

LSPAN and conversational scores were compared to examine whether higher verbal working memory is linked to stronger conversational contingency. In addition, qualitative observations were taken throughout both tasks and compared to scores to enhance understanding (Appendix D).

Participants

Participants in this research were children ages 6-12 with a confirmed diagnosis of ASD (N=10, n = 3 girls, n = 7 boys). This population was selected because the research question focuses specifically on the relationship between verbal working memory and conversational contingency within autistic communication development. This age group was chosen because children with ASD aged 6-12 years have been found to develop pragmatic and figurative language skills at a slower rate than their cognitively able peers (Kritsotakis, 2025). Additionally, this is a critical time to connect with peers, and exploring inhibitions in communication could be valuable in facilitating better social interactions for children with ASD

(Villa, 2025). Specialized schools for autism within Morris County that specifically support children with ASD were contacted to recruit participants and permission was received from two schools. Parent consent forms were also sent home to parents/guardians of eligible participants of those two schools.

Research Instruments

Data were collected using three primary instruments. Verbal working memory was measured using a modified LSPAN, with a list of sentences ranging from a 2-word to 8-word level (Appendix A). Conversational ability was assessed through a 3-minute conversation using neutral prompts listed on a sheet to elicit spontaneous speech. Participants' responses were coded using a coding scheme adapted from prior research, categorizing each turn as contingent, non-contingent, minimal, or missing (Figure 2).

Procedures

Each participant completed two sessions, spaced one week apart, individually in a room free of auditory/visual distractions. Sessions lasted approximately 10-20 minutes, with tasks kept short to reduce agitation or disinterest and ensure high-quality data. Tasks were administered in a fixed order to maintain consistency across participants, with all instructions read from a standardized script (Appendix C).

Participants received a small incentive upon completion to support engagement. This step was necessary as many children with ASD may require external incentives to engage reliably in structured tasks (Matyjek, 2023). Participants then completed the modified LSPAN followed by the 3-minute observed conversation. Throughout both tasks, qualitative observations, including behaviors from a checklist (Appendix D) and any spontaneous behaviors, were recorded. Quantitative scores and qualitative observations were later compared to identify trends, correlations, and variances between verbal working memory capacity and conversational contingency.

Results

Ten participants with ASD completed the modified Listening Span (LSPAN) task across two trials. Performance (Average LSPAN Score) was calculated as the proportion of correctly recalled sentence-final words at each level, expressed in decimal form, where 1.0 represents 100% correct. The Highest Functional Span (HFS) was also recorded, representing the highest word-level sentence at which a participant correctly recalled at least 2 final words. For example, if a participant recalled 2 out of 3 words correctly at the 5-word level but 0 out of 5 at the 6-word level, their HFS would be 5. HFS scores ranged from 2 to 8 words, and average LSPAN scores from 0.20 to 0.91 (Table 1).

Participants with the highest HFS (8-word sentences; P1, P7, P8, P9, P10) demonstrated higher recall capacity, although average LSPAN scores varied within this group (0.664 to 0.905). Participants with mid-range HFS (2-5 words; P3, P4, P6) demonstrated lower and less consistent

accuracy, while participants with the lowest HFS (two words; P2, P5) maintained low performance across both trials.

Table 1: Participant LSPAN Performance and Highest Functional Span

Participant #	Functioning Level*	Highest Functional Span (HFS)	Trial 1 LSPAN Score	Trial 2 LSPAN Score	Average LSPAN Score
P1	High	8	0.619	1.000	0.810
P2	Low	2	0.200	0.200	0.200
P3	Mixed	4	0.375	0.316	0.345
P4	Mixed	5	0.474	0.556	0.515
P5	Low	2	0.200	0.333	0.267
P6	Mixed	2	0.222	0.333	0.278
P7	High	8	0.857	0.952	0.905
P8	High	8	0.857	0.952	0.905
P9	High	8	0.600	0.7273	0.664
P10	High	8	0.630	0.720	0.675

*Functioning level reflects observed behavioral and school placement patterns, not a formal clinical diagnosis.

Trial-to-trial improvement was observed for most participants. For example, P1 performed noticeably better from scoring 0.619 in trial one to 1.0 in trial two, initially pausing to ask for repetition of sentences, but later responding more confidently and accurately. Similarly, P3 showed improved performance during trial two, suggesting that familiarity with the task and practice efforts had an influence in promoting working memory accuracy.

Participants (P1, P4, P7, P8, P10) would sometimes tend to actively reason through statements rather than simply recalling the last word. P7 and P8 consistently explained why statements were true or false before giving the final word, carefully reasoning about sentences such as “all birds fly,” showing metacognition capabilities. Participants who consistently utilized active reasoning achieved higher accuracy than peers at the same HFS level.

Semantic or schema-based errors were also common among participants. Particularly, when asked for the last word of the given statement, participants would say the subject. P3 and P4 frequently stated the subject of the sentence instead of the final word, especially at higher word levels (e.g. “fish” in response to “fish can live in trees”).

Engagement influenced performance. P2 and P5 required motivation, while P6 showed higher accuracy once rapport was established. Distractibility and impulsivity were evident in P4 and P6, who often looked away or explored their environment during the task. Despite these behaviors, both participants improved when redirected or extrinsically motivated, highlighting that focus can substantially influence working memory outcomes.

Conversational Contingency Performance

All ten participants completed the conversational contingency task across two trials. Performance was measured as the proportion of relevant (contingent) turns to total conversational turns. Scores are expressed in decimal form, where 1.0 represents 100% of turns being contingent. Average conversational contingency (CC) scores were calculated across trials for each participant (Table 2).

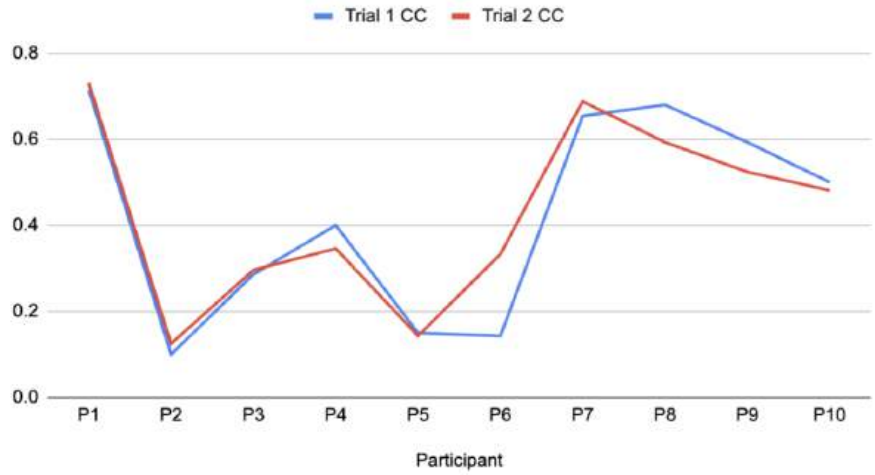
Table 2: Participant Conversational Contingency Scores

Participant #	Functioning Level	Trial 1 CC	Trial 2 CC	Average CC
P1	High	0.714	0.731	0.722
P2	Low	0.100	0.125	0.112
P3	Mixed	0.286	0.296	0.291
P4	Mixed	0.400	0.346	0.373
P5	Low	0.150	0.143	0.146
P6	Mixed	0.143	0.333	0.238
P7	High	0.654	0.688	0.671
P8	High	0.680	0.593	0.636
P9	High	0.593	0.524	0.558
P10	High	0.500	0.481	0.491

Scores ranged from low averages (P2: 0.112; P5: 0.146) to high averages (P1: 0.722; P7: 0.670). Higher-functioning participants generally demonstrated higher CC scores, while low-functioning participants scored consistently low, indicating difficulty maintaining contingent turns, which may also reflect combined effects of attention and task comprehension.

Differences between trial 1 and 2 revealed both improvements and declines (Figure 3).

Figure 3: Average Conversational Contingency Scores by Participants Across Two Trials



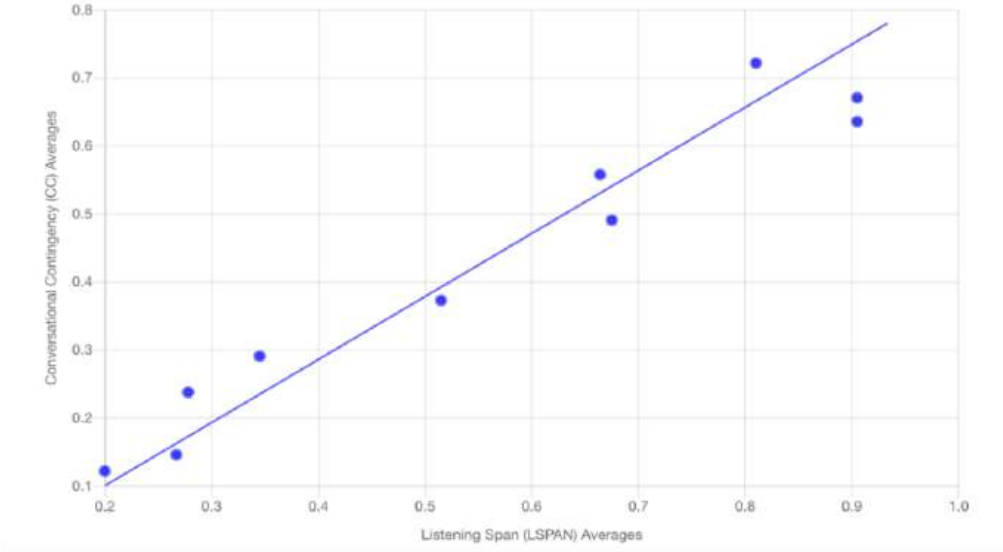
For example, P6 increased from 0.143 to 0.333, likely reflecting growing task and researcher familiarity, as well as increased engagement in the second trial. P8, in contrast, exhibited a decrease from 0.68 to 0.5926, primarily due to a rise in non-contingent elaborations.

Behavioral observations revealed additional nuances: high-scoring participants (P1, P7, P8) actively engaged and elaborated during conversations; moderate-scoring participants (P3, P4, P6) only responded contingently when personally motivated or prompted; low-scoring participants (P2, P5) relied on extrinsic motivation and often produced minimal verbal output.

Relationship Between Verbal Working Memory and Conversational Contingency

A Pearson product–moment correlation analysis was conducted to examine the relationship between verbal working memory (LSPAN average scores) and conversational contingency (CC average scores). Results revealed a statistically significant, very strong positive correlation, $r(8) = .97$, $p < .001$ (Figure 4). Such an unusually high correlation is likely inflated

Figure 4: Scatterplot displaying the relationship between Verbal Working Memory & Conversational Contingency, with line of best fit indicating a strong positive correlation



due to the small sample size and should not be interpreted as indicative of a true population-level relationship. The coefficient of determination ($r^2 = .95$) suggests that approximately 95% of the variance in conversational contingency scores is associated with variance in verbal working memory performance. However, due to the small sample size and correlational design, this value should be interpreted cautiously and cannot be generalized to the broader ASD population. Given the exploratory nature of the study and the small sample size, the correlation analysis is intended to identify potential patterns rather than establish generalizable relationships. Future studies with larger samples are necessary to determine whether this relationship remains consistent across broader groups of children with ASD.

Qualitative patterns across participants further illustrate these interactions:

- Active reasoning and elaboration: Participants who verbalized thought processes or extended responses (P1, P7) demonstrated both high LSPAN and high CC.
- Selective attention and motivation dependence: Participants with lower CC (P2, P5) often responded contingently only when personally engaged or prompted by external stimuli.
- Distractibility and impulsivity: Participants such as P6 occasionally produced non-contingent turns, despite reasonable verbal memory capacity

Overall, the data suggest a consistent pattern in which participants with higher verbal working memory scores tended to produce more contingent conversational responses.

Discussion

The findings suggest that verbal working memory may play an important role in supporting conversational turn-taking. High HFS and LSPAN scores generally were linked to higher CC, though variability reflects the multifaceted nature of conversational ability in ASD.

For example, P8, despite high LSPAN performance, produced lower CC in the second trial due to increased non-contingent elaborations, which aligns with Abbot-Smith et al. (2024)'s findings that engagement style and topic management (theory of mind), such as expanding on unrelated details, also influences conversational contingency beyond memory capacity. Active reasoning appeared to support use of memory capacity during conversation.

Conversely, low CC participants relied heavily on extrinsic motivation, highlighting the impact of engagement and attention on social communication.

The observed relationship between verbal working memory and conversational contingency aligns with the role of the phonological loop, which temporarily stores auditory-verbal information. Participants with stronger LSPAN scores likely benefited from a more efficient phonological loop, enabling them to retain prior utterances while formulating relevant responses, consistent with theoretical predictions from Baddeley and Hitch (2003).

These findings reflect Ishiguro et al. (2024), who found that working memory capacity predicted social interaction quality in adults. Similarly, ASD children in this study appeared to leverage their working memory to maintain context and respond contingently, suggesting that the role of working memory in conversation spans developmental stages.

These findings also align with prior research on ASD regarding conversational contingency and working memory. For example, Habib et al., 2019, found that children with ASD exhibit verbal working memory weaknesses that are associated with social communication difficulties. Similarly, studies have shown that differences in executive function, including attention, relate to pragmatic language performance in ASD, supporting the idea that conversational contingency is influenced by cognitive control processes (Howard et. al. 2023).

The practical implications are notable. These findings suggest that interventions aiming to improve conversational skills in ASD may benefit from targeting not only vocabulary or language, but also cognitive processes such as working memory and attention. Additionally, task

familiarity improved performance, as noticed when comparing trial performances, suggesting that structured practice can enhance both working memory and conversational contingency. By targeting cognitive processing alongside attention and social reasoning, programs can more effectively support children with ASD in developing meaningful peer interactions and long-term social competence.

Finally, the alignment between LSPAN and CC highlights the importance of mixed-methods approaches, as quantitative measures alone would not capture the nuanced effects of reasoning, engagement, and distractibility. Qualitative behavioral observations enriched the data, revealing mechanisms behind individual differences and emphasizing the value of comprehensive assessment in research and clinical practice.

Limitations

One limitation of this study is the small and highly specific participant sample. Only 10 verbal children with ASD were recruited from two specialized schools, representing a narrow subset of the broader and highly heterogeneous autism population. The New Jersey Autism Registry indicates that nearly 3.4% of children ages 1-18 are diagnosed with ASD in the county this study was based in, and the age group in this research is only a fraction of that (6-12). Additionally, by selecting only verbal participants, the study necessarily excludes nonverbal individuals or those with more severe cognitive impairments, limiting generalizability.

Recruitment challenges also contributed to this limitation. As seen in past research conducted with the ASD population, there is a significant decrease in sample size because there are accompanying challenges with ethics, trust, and participants (Gowen, 2019). Ethical requirements such as school approval and parental consent restricted the available participant pool. Additionally, parents who choose to enroll their children in research may differ from non-participants in engagement or motivation, introducing potential self-selection bias.

Another limitation relates to measurement and task constraints. Cognitive variability, attention differences, and social motivation among participants made it difficult to obtain fully consistent quantitative data. Some participants required additional prompts or incentives, and performance could fluctuate due to factors unrelated to working memory or conversational ability, such as fatigue or distractibility. Participant performance may also have been influenced by expectancy effects, as awareness of being studied or receiving incentives could have altered engagement and responses. Additionally, interpreting qualitative conversational patterns was challenging, as qualitative research involving ASD children can be unpredictable and difficult to standardize (Rasmussen, 2019). These factors emphasize the difficulty of drawing solid conclusions from small samples with high individual variability.

These limitations may partially explain variability in conversational contingency observed among participants with similar LSPAN scores, as factors such as attention and motivation likely influenced performance, showing that working memory alone does not fully account for conversational outcomes. Nevertheless, the study provides preliminary insight into how verbal working memory may relate to conversational contingency in children with ASD.

Future research with larger, more diverse populations and naturalistic observation methods would help strengthen generalizability and clarify this relationship.

Conclusion

This research provides evidence that verbal working memory is associated with conversational contingency in children with ASD. Participants with higher working memory capacity generally produced more contextually relevant turns; however, variability among participants suggest that cognitive capacity alone does not determine conversational success. Qualitative observations suggest that factors such as reasoning, engagement, and social cognition influence how working memory is utilized during communication.

These findings highlight that pragmatic language difficulties in ASD are multifaceted and may involve both linguistic and cognitive processes. Although the small sample limits generalizability, the relationship between verbal working memory and conversational contingency suggests that verbal working memory may contribute to social communication outcomes. Future research with larger, more diverse samples and naturalistic settings is needed to further examine this relationship and inform targeted interventions.

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Celiac Disease: Diagnostic Challenges, Pathogenesis, and Emerging Therapies

By Renisha Loonker

Abstract

Background: Celiac disease is an immune-mediated enteropathy triggered by dietary gluten in genetically susceptible individuals. [10,13,36,43] Although high-performance serologic assays and confirmatory histology are widely available, celiac disease remains underdiagnosed because of heterogeneous symptoms, variable testing practices, and real-world factors such as reduced gluten intake before evaluation. [7,16,23,30,43]

Objective: To synthesize current understanding of celiac disease epidemiology, pathogenesis, clinical presentation, diagnosis, and management, with emphasis on common diagnostic pitfalls and emerging directions in monitoring and adjunct therapy.

Methods: A narrative review approach was used to summarize findings from recent clinical guidelines, landmark reviews, and selected primary studies on diagnostic testing, dietary management, follow-up monitoring, non-responsive and refractory disease, and investigational non-dietary therapies. [3,4,7,10,12,16,22,30,31,41,42]

Results: Tissue transglutaminase IgA (tTG-IgA) remains the cornerstone screening test when patients are consuming gluten and do not have IgA deficiency. [3,7,10,16,23,26,36] Confirmatory testing and careful interpretation improve accuracy in borderline cases. [3,7,10,16,23,26,37] Upper endoscopy with multiple duodenal biopsies remains standard for many patients, while biopsy-sparing pathways in children can be used when strict serologic criteria are met. [3,9,11,16,18,23,26,30] Key pitfalls include false-negative serology after gluten restriction, missed IgA deficiency, patchy mucosal injury, and misattribution of symptoms to functional disorders without targeted testing. [1,3,7,16,23,26,30,50] Management relies on a strict lifelong gluten-free diet supported by dietitian education, correction of micronutrient deficiencies, and structured follow-up. [15,31,48]

Conclusions: Standardizing diagnostic pathways and reinforcing pre-test gluten exposure requirements can reduce delayed diagnosis and prevent complications. [3,7,16,23,30] Future research should prioritize accessible markers of dietary exposure and intestinal recovery, clarify the role of the microbiome, and define the clinical utility of investigational therapies as complements - not replacements - for the gluten-free diet. [12,22,28,29,41,42,44]

Keywords: celiac disease; gluten; tTG-IgA; villous atrophy; diagnosis; gluten-free diet; monitoring; microbiome; refractory disease

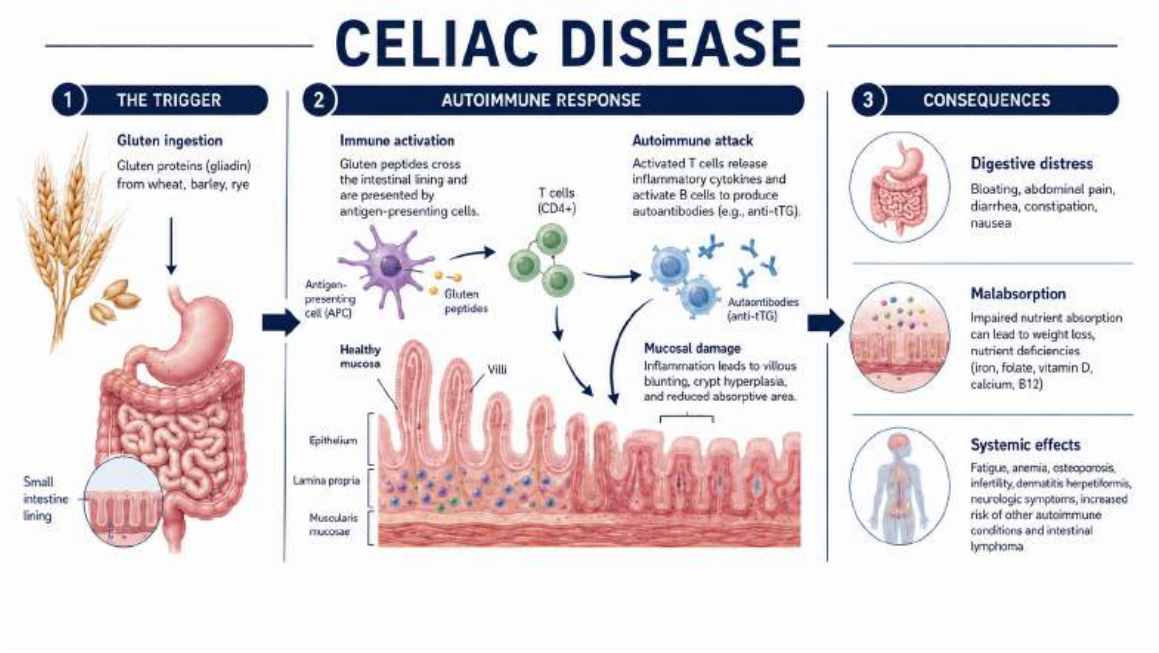


Figure 1. Overview of celiac disease triggers, autoimmune response, and consequences.

Introduction

Celiac disease is a chronic immune-mediated disorder in which ingestion of gluten (a protein complex present in wheat, barley, and rye) triggers inflammation and injury of the small intestinal mucosa. [10,13,36,43] In susceptible individuals, repeated exposure leads to characteristic villous atrophy, crypt hyperplasia, and increased intraepithelial lymphocytes, with downstream malabsorption and systemic effects. [13,20,26,36]

Although often discussed as a gastrointestinal condition, celiac disease is multisystem: it can influence growth and development in children, bone health, fertility, neurologic function, sleep and cognition, neuropsychiatric outcomes, and overall quality of life. [8,24,25,33,34,45] A major challenge is that the presentation is not uniform. Some patients have classic symptoms such as chronic diarrhea and weight loss, while others present with fatigue, iron-deficiency anemia, osteoporosis, elevated liver enzymes, dermatitis herpetiformis, or subtle nutritional deficiencies. [10,36,43,45] Many are asymptomatic and identified through screening of high-risk groups, including first-degree relatives and patients with associated autoimmune or genetic conditions. [2,35,39,40]

This variability contributes to delayed recognition and incomplete testing, even though accurate diagnostic tools exist. [7,16,23,30,43]

This manuscript reviews the epidemiologic burden and biological basis of celiac disease, the range of clinical features, and current diagnostic and management strategies. It highlights practical diagnostic pitfalls and emerging directions including improved monitoring of gluten exposure and mucosal healing, microbiome research, and investigational non-dietary therapies. [7,12,16,22,29,41,42,44]

Epidemiology and public health burden

Population studies and meta-analyses suggest that celiac disease affects roughly 1% of many populations worldwide, with estimates varying by region, age, and method of ascertainment. [38,43,47] Serology-based prevalence tends to be higher than biopsy-confirmed prevalence, reflecting both underdiagnosis and the fact that a proportion of seropositive individuals may not undergo endoscopy or have early or patchy histologic injury. [23,30,43,47]

Importantly, the gap between true prevalence and diagnosed cases remains large, which matters because prolonged untreated disease increases the risk of nutrient deficiencies, reduced bone mineral density, adverse pregnancy outcomes, and rare but serious malignancies. [24,34,45,46]

From a public health perspective, celiac disease intersects with food systems and labeling, patient education, and access to specialized dietary support. [14,15,21,32,48] Even after diagnosis, adherence is challenging: gluten is pervasive in the food supply, cross-contamination is common, and gluten-free products can be expensive. [14,21,32,49] These factors make follow-up and patient-centered counseling as critical as the initial diagnostic workup. [15,48,49]



Figure 2. Celiac disease damages intestinal villi, reducing the surface area available for nutrient absorption.

Biological basis and pathogenesis

Celiac disease develops through an interaction between genetic susceptibility and environmental exposure. [13,20,37,43] Most patients carry HLA-DQ2 and/or HLA-DQ8, which present gluten-derived peptides to CD4+ T cells in the intestinal lamina propria. Tissue transglutaminase 2 (tTG2) modifies gluten peptides through deamidation, increasing their binding affinity to HLA-DQ2/DQ8 and amplifying adaptive immune activation. [13,20,37]

In addition to adaptive responses, innate immune pathways contribute to epithelial injury. [13,20,37] Cytokine signaling, including IL-15, promotes activation and expansion of intraepithelial lymphocytes, which can directly damage enterocytes. [20,37] The resulting

mucosal remodeling produces villous blunting and crypt hyperplasia, impairing nutrient absorption. [13,20,26,36]
Autoantibodies against tTG are a hallmark of the condition and form the basis of common screening assays, but serologic positivity does not always map perfectly to symptom severity or mucosal healing. [7,10,16,31] This mismatch helps explain why symptoms and antibody titers alone can be unreliable proxies for histologic recovery. [7,16,31]

Clinical presentation, associated conditions, and complications

Clinical manifestations span classic gastrointestinal symptoms such as abdominal pain, diarrhea, bloating, and constipation, as well as extraintestinal features including iron-deficiency anemia, fatigue, short stature or delayed puberty, osteoporosis, elevated transaminases, peripheral neuropathy, migraines, infertility, and dermatitis herpetiformis. [10,24,25,34,36,43,45] Children more often present with growth failure or gastrointestinal complaints, whereas adults frequently present with extraintestinal findings or nonspecific symptoms. [9,11,18,36,43]
Celiac disease is also associated with other autoimmune conditions and genetic syndromes, which motivates targeted testing in high-risk groups. [2,35,39,40,45] Examples include type 1 diabetes and autoimmune thyroid disease, as well as first-degree relatives of affected individuals. [35,39,40] In these settings, clinicians may have a lower threshold to test even in the absence of classic gastrointestinal symptoms. [3,10,16,30]

Delayed diagnosis is clinically important. Chronic inflammation and malabsorption can contribute to micronutrient deficiencies and impaired bone health. [15,34,45,48] A subset of patients has ongoing symptoms despite a gluten-free diet due to continued exposure, overlapping disorders such as lactose intolerance during mucosal recovery, irritable bowel syndrome, or small intestinal bacterial overgrowth, or less common entities such as refractory celiac disease. [1,31,50] Rare complications include enteropathy-associated T-cell lymphoma (EATL), which is strongly linked to long-standing inflammation and refractory disease. [31,45]

Methods

This manuscript is written as a narrative review. Core themes were selected based on relevance to real-world diagnosis and management: serologic testing, duodenal biopsy and histology, biopsy-sparing diagnostic pathways, genetic testing, gluten challenge, follow-up monitoring, non-responsive and refractory disease, and emerging directions including microbiome research and investigational non-dietary therapies. Sources emphasize clinical guidelines, high-quality review articles, and selected primary studies.

Results and synthesis

Table 1 summarizes common diagnostic tools and interpretation issues emphasized across clinical guidelines, diagnostic accuracy reviews, and current management papers. [3,4,7,10,16,23,26,30,36]

Tool	What it tells you	Common pitfalls / notes
tTG-IgA + total IgA	Preferred first-line serology in most patients on a gluten-containing diet	False negatives if gluten restricted or IgA deficient; weak positives need confirmation
EMA (IgA)	Highly specific confirmatory test; used in some biopsy-sparing pediatric pathways	More operator-dependent; typically used after positive tTG
DGP (IgG/IgA)	Helpful in IgA deficiency or young children in some settings	Interpret alongside clinical context and other serology
Upper endoscopy with multiple duodenal biopsies	Confirms villous injury; assesses differential diagnoses	Patchy disease - take multiple samples from bulb and distal duodenum
HLA-DQ2/DQ8 typing	High negative predictive value when absent	Positive result is common and not diagnostic by itself

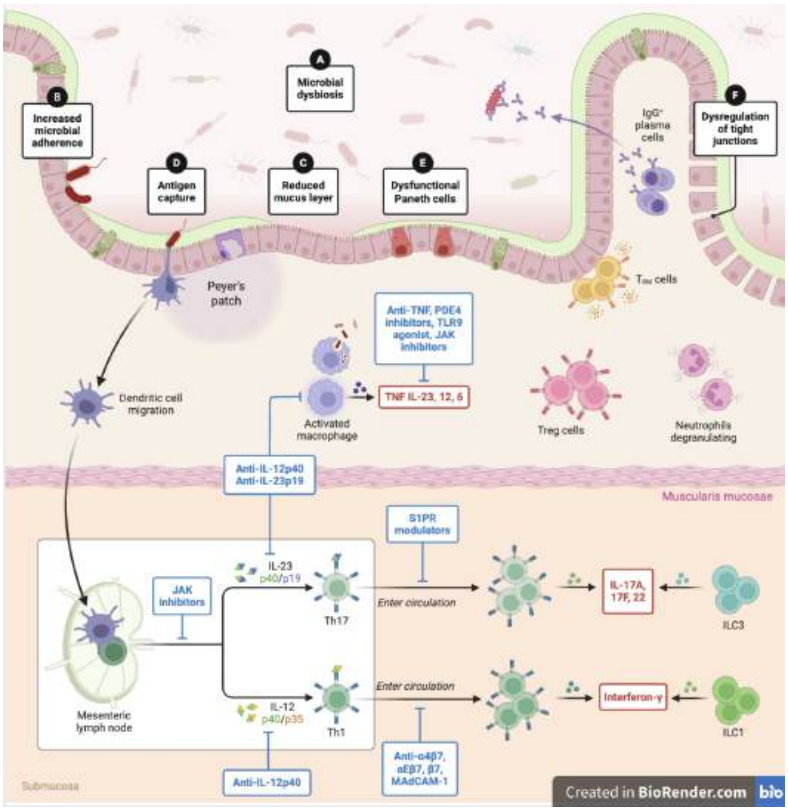


Figure 2. Immune pathways and therapeutic targets in celiac disease.

Serologic testing

The preferred initial serologic test for most patients is tTG-IgA, typically paired with a total serum IgA level to identify IgA deficiency. [3,7,10,16,23,26,36] When IgA deficiency is present, IgG-based assays, including tTG-IgG or deamidated gliadin peptide (DGP) IgG, are used. [3,10,16,23]

Endomysial antibody (EMA) testing is highly specific and is often used as a confirmatory test when tTG-IgA levels are elevated or when a biopsy-sparing pathway is being considered in pediatric practice. [7,9,11,16,18,23]

A key practical issue is that many patients reduce or eliminate gluten before testing, especially if they have already experimented with diet changes. [3,7,16,30] Even partial gluten restriction can lower antibody titers and increase false-negative results. [3,7,16] For accurate interpretation, testing should occur while the patient is consuming gluten in adequate amounts; if a patient has already started a gluten-free diet, clinicians may consider a supervised gluten challenge based on guideline recommendations and clinical context. [3,7,16,30]

Serology is also used in follow-up, but normalization of antibodies does not guarantee mucosal healing. [7,16,31]

Duodenal biopsy and histology

Upper endoscopy with duodenal biopsies remains a central confirmatory tool in many diagnostic pathways, particularly in adults. [3,10,16,23,26,30,36] Histologic findings include increased intraepithelial lymphocytes, crypt hyperplasia, and varying degrees of villous atrophy. [13,20,26,36]

Because mucosal changes may be patchy, obtaining multiple samples from both the duodenal bulb and distal duodenum improves diagnostic yield. [16,23,26,30]
Histology is not perfectly specific. Infections, medication effects, inflammatory conditions, and other causes of enteropathy can mimic aspects of the celiac pattern. [3,16,26,30] As a result, biopsy findings are best interpreted alongside serology, diet history including gluten exposure, and clinical features. [3,16,23,26,30]

Many centers use a modified Marsh classification to describe the spectrum of injury, from increased intraepithelial lymphocytes with preserved villous architecture to partial or total villous atrophy. [3,26,36]

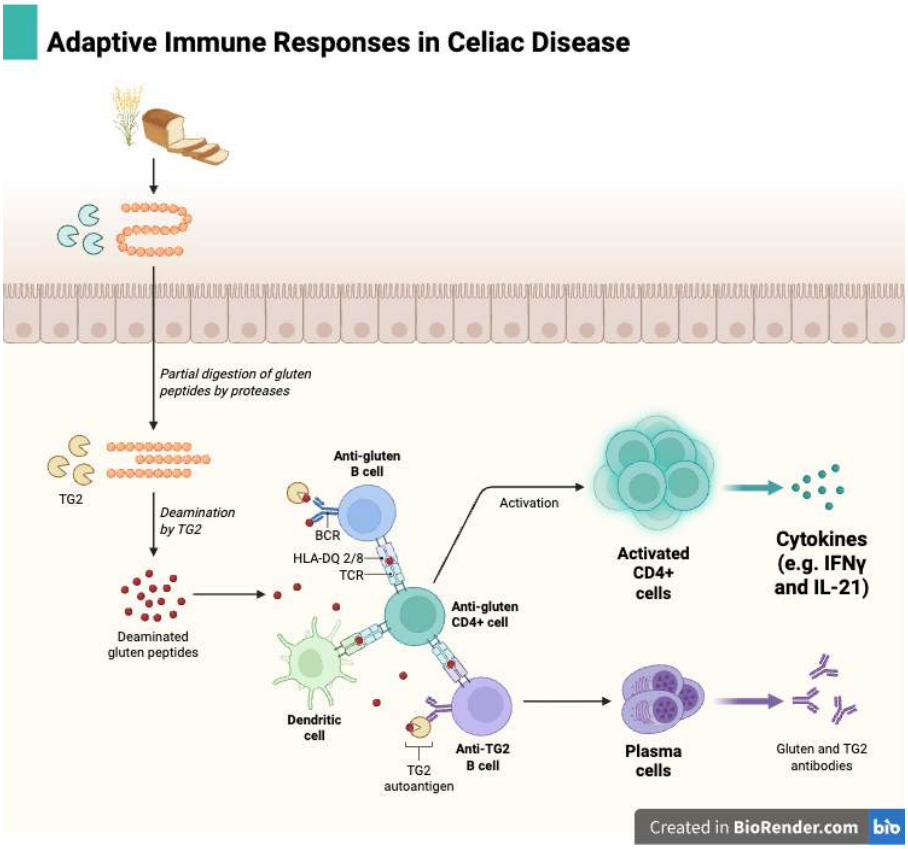


Figure 3. Adaptive immune responses in celiac disease.

Biopsy-sparing pathways and genetic testing

Pediatric guidelines allow diagnosis without biopsy in carefully selected cases, typically when tTG-IgA levels exceed a high threshold and are confirmed by a second serologic test, often EMA, alongside consistent clinical presentation. These criteria aim to preserve diagnostic accuracy while reducing invasive procedures. [9,11,18]

In adults, biopsy-sparing approaches are less standardized and remain an area of active research and practice variation. [3,16,30]

HLA typing has high negative predictive value: absence of HLA-DQ2 and HLA-DQ8 makes celiac disease unlikely. [7,10,16,26,36,37] However, these alleles are common in the general population, so a positive result alone is not diagnostic. [7,16,26,36] HLA testing is most useful in equivocal cases, in patients already on a gluten-free diet, or when prior testing results are incomplete or discordant. [3,7,16,26,30]

Management: gluten-free diet, nutrition, and support

The cornerstone of treatment is a strict lifelong gluten-free diet, ideally implemented with guidance from a registered dietitian experienced in celiac disease. [10,15,48] Avoidance includes wheat, barley, and rye, and requires attention to hidden sources of gluten and cross-contamination in shared kitchens, restaurants, and manufacturing. [10,15,21,32,48,49] In

the United States, foods labeled gluten-free must contain less than 20 parts per million of gluten, a threshold generally considered safe for most patients. [14,48]

Nutritional assessment is essential at diagnosis and during follow-up. [15,48] Common deficiencies include iron, folate, vitamin B12, vitamin D, and calcium; supplementation is individualized based on laboratory findings. [15,48] Bone mineral density testing is often considered in adults or in those with risk factors for osteoporosis. [34,48] For dermatitis herpetiformis, symptom control may require short-term medication while the gluten-free diet gradually reduces immune activity. [10,36,43]

Because adherence can be difficult, patient education, support groups, and practical skill-building such as label reading, meal planning, and safe dining strategies are important components of care. [15,21,32,48,49]

Gastrointestinal	Extraintestinal	Associated conditions / higher-risk groups
Diarrhea, steatorrhea, abdominal pain, bloating, constipation	Iron-deficiency anemia, fatigue, osteoporosis/osteopenia, elevated transaminases	First-degree relative with celiac disease; type 1 diabetes; autoimmune thyroid disease
Weight loss, poor appetite, nausea	Dermatitis herpetiformis, aphthous ulcers, neuropathy, migraines	Selective IgA deficiency; Down syndrome; Turner syndrome
Growth failure, delayed puberty (pediatrics)	Infertility, adverse pregnancy outcomes, depression/anxiety	Unexplained anemia, osteoporosis, persistently elevated liver enzymes

Table 2 summarizes symptoms, extraintestinal manifestations, and higher-risk groups described in clinical reviews and condition-specific meta-analyses. [2,8,24,25,34,35,36,39,40,43,45]

Follow-up monitoring, persistent symptoms, and refractory disease

Follow-up typically includes assessment of symptom response, repeated serology, evaluation for nutritional deficiencies, and review of diet adherence with a dietitian. [15,31,48] Antibody titers often decrease over months on a strict gluten-free diet, but the pace of decline varies. [7,16,31]

When symptoms persist, clinicians evaluate for inadvertent gluten exposure as well as overlapping conditions such as lactose intolerance, pancreatic insufficiency, microscopic colitis, or irritable bowel syndrome. [1,31,50]

Refractory celiac disease is defined by persistent or recurrent malabsorptive symptoms and villous atrophy despite adherence to a gluten-free diet after other causes have been excluded. [31,45] It is uncommon but clinically important due to risks of severe malnutrition and progression to lymphoma, particularly in type II refractory disease characterized by abnormal or clonal intraepithelial lymphocytes. [31,45]

Management generally requires specialist care, careful nutritional support, and immunomodulatory therapy tailored to disease subtype. [31,45]

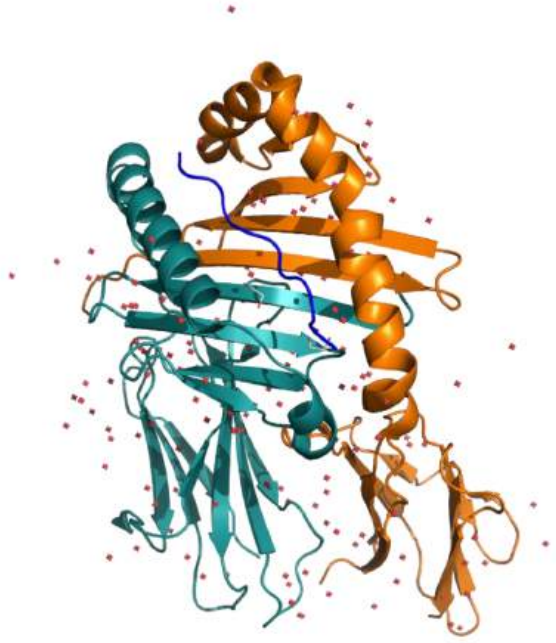


Figure 4. X-ray structure of HLA-DQ2.5-glia-α1 complex

Emerging directions: biomarkers, microbiome, and adjunct therapies

A central research gap is monitoring. Symptoms and serology do not always align with mucosal healing, yet repeat endoscopy is burdensome. [7,16,31] As a result, there is interest in adjunct biomarkers that reflect recent gluten exposure or intestinal recovery. [7,16]

Approaches under study include assays that detect gluten immunogenic peptides in stool or urine and refined serologic or inflammatory markers that may better correlate with histologic status. [7,16,28]

The gut microbiome is also actively studied as a potential contributor to disease risk, symptom profiles, and response to dietary treatment. [29,44] Studies have reported differences in microbial composition and function in patients with active celiac disease compared with controls, but findings vary across cohorts and methods. [29,44] It remains uncertain whether microbiome shifts are a cause, a consequence, or a modifier of disease. [29,44]

Because a gluten-free diet is difficult to maintain perfectly and may not fully resolve symptoms for all patients, multiple adjunct therapies are under investigation. [12,22,28,41,42]These include enzymes designed to degrade gluten, agents that reduce intestinal permeability, immune-targeting approaches, and strategies to neutralize key pathogenic pathways. [12,22,41,42]

For example, selective oral transglutaminase 2 inhibitors have shown proof-of-concept reduction of gluten-induced mucosal injury during controlled gluten challenge in clinical studies. [12,22,41,42] At present, such approaches remain investigational and should be viewed as potential complements to - not replacements for - dietary therapy. [12,22,28,41,42]

Discussion

Timing	Clinical focus	Common labs / assessments
3-6 months	Symptom review; diet adherence; address cross-contamination	tTG/EMA (as used); CBC; iron studies if deficient
6-12 months	Nutritional optimization; manage persistent symptoms	Vitamin D, folate, B12; CMP/LFTs if abnormal
Annually	Long-term prevention; quality-of-life; comorbidity screening	Repeat serology; consider DEXA in at-risk patients

Table 3 summarizes a practical follow-up framework based on nutrition practice guidance and refractory disease management recommendations. [15,31,48]

Celiac disease illustrates how a single dietary exposure can drive a complex immune response with systemic consequences. [13,20,37] Clinically, the major barrier is not the absence of diagnostic tools, but inconsistent application of those tools in the context of variable symptoms and real-world behaviors such as gluten reduction before evaluation. [3,7,16,30]

Standardized diagnostic pathways that account for IgA deficiency, ensure adequate pre-test gluten exposure, and optimize biopsy sampling can reduce missed diagnoses. [3,7,16,23,26,30]

A second gap is long-term monitoring. Accessible markers of gluten exposure and mucosal healing would strengthen both clinical care and research by reducing reliance on repeat endoscopy and enabling more objective assessment of adherence. [7,16,31] In parallel, microbiome studies and emerging therapies represent promising areas, but they require careful study design, standardized endpoints, and long-term safety evaluation, especially given that many patients can achieve excellent health outcomes with a well-supported gluten-free diet. [12,22,29,41,42,44,48]

Limitations

This manuscript is a narrative review and does not include a systematic search or formal risk of bias assessment. Conclusions should be interpreted as a synthesis of current themes rather than a quantitative meta-analysis. Future versions could strengthen rigor by specifying databases searched, inclusion criteria, and structured study quality.

Conclusion

Earlier recognition of celiac disease depends on awareness of non-classic presentations and consistent use of evidence-based diagnostic pathways. [3,10,16,30,36,43,45] While the gluten-free diet remains the foundation of treatment, the field is moving toward better tools for monitoring exposure and healing, as well as therapies for patients who continue to struggle with symptoms or complications. [7,12,16,22,28,31,41,42]

Continued research integrating immunology, nutrition, and microbiome science is likely to refine diagnosis and expand treatment options. [12,20,29,37,41,42,44,48]

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Literature Review of Early Indicators of CTE and Head Trauma in High School and College-Aged Football Players By Michael Dinevski

Abstract

There is growing evidence that repeated brain injuries sustained while playing high school and college-level football, including sub-concussive impacts, may contribute to early signs of the neurodegenerative disease known as Chronic Traumatic Encephalopathy (CTE). Currently, because it is only possible to detect CTE post-mortem, there is a need to identify potential early markers of trauma-induced neurodegeneration among young athletes. This review focuses on the effects of early repeated head trauma on high school/college-level athletes and its correlation to early markers of Chronic Traumatic Encephalopathy (CTE).

CTE is a neurodegenerative disease that develops over time and causes changes in functioning, cognition, and behavior. The evolution of CTE occurs in four stages, each presenting unique and specific symptoms (McKee, 2013):

Stage I	Asymptomatic, or mild memory and depressive symptoms.
Stage II	Symptoms include behavioral outbursts and severe depression
Stage III	Cognitive deficits including memory loss and executive dysfunction
Stage IV	Advance language deficits, psychotic symptoms, profound cognitive deficits, and motor features.

Table 1: The cognitive and behavioral symptoms corresponding to each stage of CTE, according to a study published by McKee (2013).

Methods

This review combines peer-reviewed papers published over the last 10 years through online databases such as PubMed and Google Scholar. It is focused on three primary areas: structural and neuroimaging changes, identification of blood biomarkers, and cognitive outcomes associated with cumulative exposure to head impacts.

Results

There is a substantial body of evidence suggesting that alterations in brain structure, most notably white matter degeneration measured by fractional anisotropy (FA), and the presence of various fluid biomarkers (p-Tau, UCH-L1, GFAP, and NfL) can occur in young athletes after experiencing frequent injuries to the head. In addition, metrics that take cumulative exposure into account, like the Cumulative Head Impact Index (CHII), have a strong non-linear, dose-response correlation with the chances of developing cognitive and behavioral disorders.

Conclusion

Only going off of a concussion diagnosis is not enough to evaluate the chance of neurological disorders developing in the future. Comparing neurocognitive tests to baseline scores, cumulative impact monitoring through equipment-bound accelerometers, and biomarker analysis can act as early indicators of neurodegeneration in athletes and allow preventive intervention early in the disease's progression.

The main aim of this narrative review is to compile previous studies on early evidence of trauma-induced neurodegeneration among young athletes. This paper looks into the mechanisms of repeated head injury, discusses early structural and functional changes in the brain, and analyzes the effectiveness of biochemical indicators. Additionally, this review will describe the dose-response relationship between cumulative head impacts (CHI) and cognitive dysfunction, and assess ethical and policy issues in youth contact sports.

1. Introduction

There is a growing concern over the long-term neurological damage correlated with youth contact sports, leading to much scrutiny and criticism of the sports themselves. One sport, in particular, that is receiving such backlash is youth-level American football. What's crucial to understanding this concern is that head trauma-related complications are not just limited to professional athletes; evidence suggests that neurological changes can accumulate much earlier in an athlete's career. A study published in the AAP's journal Pediatrics estimated that a total of 1.1 to 1.9 million sports and recreation-related concussions (SSRCs) occur annually among the 44 million youth athletes in the United States; a significant portion of these injuries (511,590 to 1,240,972 athletes) was not seen in health care settings (Bryan et al., 2016). Additionally, it is vital to mention that sub-concussive impact (impacts that cause movement of the brain but do not result in immediate clinical symptoms) carries a cumulative threat over time; there is evidence suggesting that subconcussive-level impact can result in neurological changes, especially after experiencing these blows to the head repetitively (Johnson et al., 2014).

Closely associated with repeated head injuries is a degenerative neurological condition known as Chronic Traumatic Encephalopathy (CTE). CTE is a progressive and degenerative brain disorder. The causes of the condition include both concussive and sub-concussive hits ("Frequently Asked Questions," 2019). The condition is progressive, meaning it develops over time and eventually leads to complete deterioration of brain function ("Chronic Traumatic Encephalopathy: Symptoms," 2023). Some of the symptoms include memory loss, confusion, mood disorders (such as depression, anxiety, and irritability), behavioral problems (like impulsivity and aggressiveness), and motor problems ("Chronic Traumatic Encephalopathy FAQs," n.d.). Currently, there is no proven cure, and the only way to diagnose it is after the death of the patient ("Chronic Traumatic Encephalopathy: Diagnosis," 2019).

In 2017, Mez et al. conducted an important study looking into the brains of deceased ex-football players. The study found certain indicators of CTE in 21% of players who played at the high school level and 91% of players who played at the university level. This is in contrast with the results obtained from professional-level players, where CTE was found in 99% of

former NFL players studied (Mez et al., 2017). The study shows that CTE exists even in cases of high school players, meaning this disease is not limited to athletes who played football at the professional level (Mez et al., 2017).

Current research now revolves around determining early cognitive and psychological changes to the brain that could serve as a sign of long-term brain damage (Didehbani et al., 2020). Recent research suggests that early biological markers, such as abnormal Tau protein accumulation detected via blood tests, may indicate CTE risk (Miner et al., 2024), as well as white matter changes detected through neuroimaging techniques (Kuzminski et al., 2018), may indicate CTE risk. Such indicators are very important in the prevention of CTE's progression in younger athletes.

2. Methods

This paper used peer-reviewed literature accessed through PubMed and Google Scholar. The methods of searching for academic literature involved two phases: first, a search was used to find published literature relating to the search terms "chronic traumatic encephalopathy," "subconcussive impact," "youth football head trauma," "Tau biomarkers," "cumulative head impact index," and "white matter football." Second, the search was limited to studies conducted within the last 10 years, and studies that did not limit their sample demographic to professional athletes alone, thus focusing on studies that also included high school and college athletes as the primary focus area of the current research. The contents of the research papers that were selected for this review were focused on the following areas: a) structural/neuroimaging changes; b) blood biomarkers; and c) cognitive outcomes over time. In addition to the epidemiological studies used in the paper (e.g., concussion prevalence data), studies describing the pathophysiological mechanism of CTE were used as well. Attention was paid to studies published in peer-reviewed journals versus non-peer-reviewed journals.

3. Results and Discussion

3.1: Overview of Repeated Head Trauma in Football

A. Mechanisms of Head Trauma

Repeated head trauma is common among football players across all age groups during their seasons (Broglio et al., 2011). In terms of these impacts, there are two types of head injuries: concussive head injuries and subconcussive head injuries("Frequently Asked Questions," 2019). A concussion produces observable symptoms, such as headaches, confusion, and problems maintaining balance, whereas subconcussive head injuries are head injuries that do not produce any recognizable symptoms (Cleveland Clinic, 2020). It is not the big-hit concussions but rather the accumulation of many sub-concussive hits to the head and body that increases the likelihood and extent of CTE (Tagge et al., 2018). According to a study conducted

by Stein et al. (2015), 16% of people with CTE identified after death never had a single concussion in their life.

It is especially worrying how common such hits can be in young football players. Research was carried out that assessed head impact exposure in youth football. The impacts were measured using 12 accelerometers installed in the helmets of seven 7- and 8-year-old players throughout the season. In total, 748 impacts were measured during the season from the 7 players involved, averaging 107 impacts per player (Daniel et al., 2012). The linear acceleration of these hits reached 100g (Daniel et al., 2012). Research done by Guskiewicz et al. (2007) found that "concussions were recorded ranging in impact magnitudes of 60.51 to 168.71 g". This shows that these 7- and 8-year-old children are experiencing hits reaching linear accelerations within the likely range for concussions, and with the 107 impacts the child experiences every season, it is very possible that one of the hits reaches this acceleration threshold.

B. Measurement Tools

To study and measure the structural changes caused by repeated blows to the head, scientists have begun to employ modern neuroimaging techniques. One technique is the use of MR imaging of the players' heads before and after the sports season. Seventeen male athletes (mean age = 16 ± 0.73 years) underwent MR imaging before and after the season. Changes in fractional anisotropy across the white matter structure were then assessed in the study from the start of the season compared to the images at the end of the season. These results showed significant ($P < .05$, corrected) decreases in fractional anisotropy in the fornix-stria terminalis, and cingulum hippocampus, with FA negatively correlated with impact frequency. The fornix, stria terminalis, and cingulum hippocampus are three important white matter fiber pathways that form a network that converts emotions into memory, physiological actions, and consciousness. The effects were strongest in the fornix-stria terminalis, where decreases in fractional anisotropy were correlated with worsening visual memory (Kuzminski et al., 2018). Fractional anisotropy (FA) shows whether water molecules diffuse more in one direction than another, which is related to the organization and integrity of tissue structures like nerve fibers in the brain: densely packed, well-myelinated, and well-aligned axon bundles cause water diffusion into one dominant direction, resulting in high FA scores, while damaged and unstructured axon bundles allow water diffusion in all directions, resulting in low FA scores (Kochunov et al., 2012). Low FA scores, therefore, indicate degeneration of axonal structures and damage to the white matter structure (Kraus et al., 2007). According to Kuzminski et al. (2018), FA scores significantly decrease during a single season of high school football play and correlate with the number of impacts, suggesting the presence of white matter damage even in the absence of concussions due to repetitive sub-concussive impacts.

Along with neuroimaging, researchers have begun to use head impact sensors, linear and rotational accelerometers, and angular rate sensors to study different aspects of head motion during impact. Daneshvar et al. (2023) compared how well different models predict CTE

diagnosis and severity (Table 2):

	Odds ratio (95% CI)	*p*-value	BIC	Cross-validation mean error	AUC
Concussion number	1.00 (1.00–1.00)	0.23			
Position, non-speed vs speed[b]	1.39 (0.89–2.16)	0.15			
Duration of play per year	1.15 (1.11–1.19)	9.4×10^{-13}	685.3	0.177	0.716
CHII per 1,000 hits	1.21 (1.13–1.29)	7.6×10^{-9}	705.0	0.183	0.698
CHII-G per 10,000 g	1.20 (1.15–1.25)	2.0×10^{-15}	665.9	0.170	0.757
CHII-R per 1,000,000 rad/sec^2	1.22 (1.16–1.29)	7.0×10^{-15}	665.2	0.169	0.763

Table 2: The values show the accuracy of models in predicting CTE, comparing the number of concussions, player position, duration of play, CHII per 1000 hits, and Cumulative linear (CHII-G) and rotational acceleration (CHII-R). The BIC stands for Bayesian Information Criterion and AUC stands for Area Under Curve.

- Odds Ratio: Demonstrates how closely an occurrence (developing CTE) is correlated with a particular measured variable (number of concussions, position, duration of play per year, etc). If OR is 1.00, then the study concluded that the variable does not change the likelihood of getting CTE. If OR>1.00, then the study concluded that as the measured variable increased, so did the odds of getting CTE.
- p-value: Shows whether this odds ratio is statistically significant or not. Values below 0.05 are generally considered significant (the correlation between the measured variable and diagnosis of CTE was likely not due to random chance); any values above it imply a variable's correlation to CTE likely occurred by random chance.
- AUC (Area Under the Curve): Shows how well each variable distinguishes between players who have CTE and players who do not have CTE. 0.5 means the variable is no better than random guessing, while 1.0 implies the variable can accurately distinguish between CTE and non-CTE cases every time. A higher AUC is better.

Neither the number of diagnosed concussions nor player position was found to be a significant predictor of CTE (OR=1.00, p = 0.23, p=0.15 respectively). In contrast, cumulative exposure measures were found to be predictive, and CHII-G (cumulative linear acceleration) and CHII-R (cumulative rotational acceleration) displayed the highest predictive accuracy with respective AUC values of 0.757 and 0.766, significantly more accurate than the duration of play model ($p = 9.5 \times 10^{-5}$ and $p = 1.5 \times 10^{-5}$ respectively) (Daneshvar et al., 2023). These results indicate that traditional concussion counting is an unreliable measure of neurological risk, while cumulative exposure measures based on acceleration may be more reliable.

Researchers have also started to use mouthpieces as tools for measuring impact. Since mouthguards are much closer to the skull than the sensors placed on helmets, they could result in more accurate measurements of impact (Liu et al., 2020).

3.2. Early Effects on Brain Activity in High School and College Players

A. Structural Changes

Repeated exposure to physical trauma can also alter brain structure; specifically, a study by Miner et al. (2025) found substantial changes in white matter using diffusion tensor imaging (DTI) and MRI. The same study also hypothesizes that RHI (repeated head injury) leads to long-term white matter changes that “manifest in a unique pattern” on MRI imaging. Such changes have also been linked to behavioral and cognitive problems later on in life (Stamm et al., "Age of First Exposure", 2015).

Repeated blows to the head can harm white matter's ability to transmit signals from one gray matter area of the brain to another, leading to cognitive dysfunction (Hebert and Filley, 2022).

In an investigation of the brain tissue of 205 former football players, Alosco et al. (2023) noted that individuals who had played football for longer and started taking tackles earlier in their playing career had less of the critical proteins proteolipid protein 1 and myelin-associated glycoprotein in their frontal white matter and worse self-reported executive function and impulsivity. There is a clear dose-response: the more a given individual is repeatedly exposed to the stress of concussive and sub-concussive hits, the greater the injury and the more severe the behavioral or cognitive consequences. Repeated exposure to traumatic brain injury can also cause brain region shrinkage. Neuropathological evidence of CTE can be detected in the brains of players from all positions who have been studied post-mortem, including a reduction in the volume of the hippocampus (a part of the brain used for memory formation) (Khan and Talley, 2025).

These findings suggest that CTE has significantly contributed to the memory loss, especially, episodic memory loss, reported by individuals after years of repeated head trauma during contact sports.

B. Functional and Cognitive Changes

An analysis of eleven male athletes between the ages of 15 and 19 showed that even though the players did not exhibit concussion symptoms, they suffered from changes in neural activity in the DLPFC and impairments in their visual working memory, as reported by Talavage et al. (2014). Neurocognitive functioning was evaluated using ImPACT tests (which determine cognitive performance such as attention span, working memory, sustained attention, and selective attention). Brain activity was observed using fMRI, focusing on the participant’s dorsolateral prefrontal cortex (DLPFC). This area of the brain is important for functions such as execution and cognitive control (Friedman and Robbins, 2022). This study shows that a person

may suffer from brain complications even if they have no noticeable symptoms after sustaining many blows to their head. From this, you can see that the most significant cognitive and functional changes in a person that can be noted from CTE are: problems with memory, difficulty in organizing, planning, and solving problems, problems with understanding and communicating, issues with balance and movements, uncontrollable shaking, and inappropriate behavior.

C. Biochemical Markers

High school and college football players also show increased levels of certain biomarkers after experiencing RHI. These biomarkers appear in the player's blood samples (Papa et al., 2022). These biomarkers include:

- **Tau Protein:** The major feature of Tau protein as a biomarker is the presence of abnormally collected Tau protein (p-Tau). The p-Tau clumps together to form neurofibrillary tangles (NFTs), which harm normal neuronal function and may lead to cell death.
- **Ubiquitin C-terminal hydrolase L1 (UCH-L1):** released by damaged neurons, it can be found in cerebrospinal fluid and blood
- **Neurofilament light chain (NfL):** the NfL gets released into the extracellular space (including CSF and blood) when axons get damaged
- **S100B:** S100B can be transferred into blood due to damage to the blood-brain barrier

Researchers conducted a study of 11 varsity high school football players who experienced head collisions. The players wore helmets with accelerometer sensors that relayed information to a computer. The researchers in this study found significant levels of ubiquitin C-terminal hydrolase L1 (UCH-L1) and Tau protein, which are signs of neuronal injury and axonal injury, respectively (Joseph et al., 2019). These biomarkers are not proof of CTE, but they are indicators of brain injury. Because of this, elevated levels of these proteins are found in CTE patients, but are also found in patients with other brain injuries, such as stroke.

3.3: Early Markers of Chronic Traumatic Encephalopathy (CTE)

A. Tau Protein Accumulation

This repeated trauma to the head results in a cascade of damage to the brain's chemical processes. Tau is a natural protein that provides stability to the internal structure of cells, including maintaining the shape and function of microtubules, which are an important part of the cytoskeleton. With each impact, sudden stretching and twisting of the cell membranes occur (Ahmadzadeh et al., 2014). The result of this is a huge flow of calcium into the cells (Lingor et al., 2012). The influx of calcium leads to a substantial disruption of the chemical composition of enzymatic processes:

1. Enzymes called kinases (GSK3beta and CDK5) are overactivated (Mahaman et al., 2019).
2. Phosphatases responsible for cleaning the cells are inhibited (Baltissen et al., 2023).

As a result of these events described above, too many phosphate molecules attach to the Tau protein, causing the protein to become hyperphosphorylated. A hyperphosphorylated simply means that the protein has an abnormally high number of phosphate groups attached to it. This hyperphosphorylation can lead to dysfunction in the protein, causing it to fold and form tangles or clumps (Murray et al., 2014). The pattern of Tau tangles in CTE is very distinct and specific compared to other diseases, helping researchers distinguish the disease from Alzheimer's and other neurodegenerative diseases (Stein et al., 2014). The abnormal Tau deposits (called p-Tau protein) in CTE are often around small blood vessels in the cortical sulci (the grooves or depressions on the surface of the cerebral cortex) (Butler et al., 2022). The accumulation of Tau tangles disrupts normal nerve cell communication, leading to cell damage and eventual cell death (James et al., 2015). By identifying this abnormal accumulation of Tau protein during post-mortem examination, researchers can definitively diagnose CTE. It is still not possible to identify this CTE-specific tau pattern in patients who are alive because the abnormal proteins are very small and dispersed among the deep grooves of the brain. Current scanning techniques, like CT scans and MRIs, lack the necessary resolution(Alosco et al., 2021). Still, the Tau concentration in a blood sample would show useful signs of neurological damage in general.

B. CTE Diagnosis Challenges

In the past, observing this unique p-tau formation during an autopsy was the only reliable method for diagnosing CTE (Pierre et al., 2021). This is why finding dependable in vivo diagnostic techniques has become a priority for researchers. Although some fluid biomarkers can indicate levels of certain proteins in the bloodstream, they only indicate general neurological damage or brain injury. While a blood test can indicate high tau levels in the blood that signal neurological distress, it cannot show where tau has accumulated in the unique sulci pattern of CTE (Pierre et al., 2022). The complications with diagnosing CTE in living patients also stem from overlapping symptoms. For example, episodic memory loss, changes in mood, decline in cognitive abilities, etc., are signs of many other conditions, like dementia, Alzheimer’s disease, or Parkinson’s disease (Adams et al., 2018). Though practitioners can arrive at a likely diagnosis of CTE by evaluating a person's history of repeated head trauma and symptoms, it is still difficult due to CTE’s overlap with the symptoms of other neurodegenerative/brain diseases.

C. Emerging Early Markers

Though neither the imaging techniques nor the biomarkers mentioned above are capable of distinguishing between CTE and other types of tauopathy, future research could use machine learning methods to distinguish between tau accumulations detected by PET/fMRI in patients suffering from Alzheimer's disease and CTE (Bergauer et al., 2022).

One recent clinical trial utilized the tau-specific PET tracer Flortaucipir to perform imaging of tau in athletes who have sustained repeated head trauma during contact sports. Results from this initial work showed a larger amount of tau in CTE-related brain areas in former athletes with repetitive head injuries in comparison to controls (Stern et al., 2019).

While these results seem promising, Flortaucipir currently does not demonstrate the sensitivity necessary for differentiating between stages of traumatic encephalopathy syndrome (TES) (Su et al., 2023). Also, since the tracer was developed to study Alzheimer's disease, it produces "off-target binding" which occurs when the tracer attaches to iron deposits and neuroinflammatory processes instead of tau protein itself (Baker et al., 2019). This can create false positives unrelated to CTE.

Although tau PET imaging is a useful research approach, it cannot be used as an independent tool for diagnosing CTE. It is still necessary to rely on a multi-level approach through fluid markers, imaging data, and early symptoms of the disease, including impulsivity, mood problems, and deficits in thinking.

3.4: Relationship Between Youth Head Trauma and CTE

A. Evidence from Longitudinal Studies

Stamm et al. (2015) conducted a study on forty-two former National Football League (NFL) players ages 40–69 from the Diagnosing and Evaluating Traumatic Encephalopathy using Clinical Tests study (a study used to diagnose CTE). Subjects were divided into 2 groups based on their age of first exposure (AFE) to tackle football: AFE <12 and AFE ≥12. The subjects then took multiple tests (WCST, NAB-LL, and WRAT-4 Reading tests) to measure their executive function, memory, and cognitive flexibility. The study found that former NFL players in the AFE <12 group performed significantly worse than the AFE ≥12 group on all measures of the cognitive tests. The study also reported that the tests were controlled for the number of years played and the age of the subjects (Stamm et al., "Age at First Exposure", 2015). This study shows that the age of first exposure to repeated head trauma is a huge factor in the degree of cognitive impairment experienced by individuals: experiencing TBIs earlier in life was correlated with a greater decrease in cognitive functioning, memory, and flexibility. It can also be inferred that experiencing repeated head impacts during a critical developmental period may increase the risk of cognitive impairment later in life.

B. Dose-Response Relationship

Research has also suggested a dose-response relationship between cumulative head impact exposure and later-life cognitive impairment. A study conducted by Montenigro et al. (2017) on 93 former high school and collegiate football players was used to measure the correlation of cumulative exposure to repetitive head impacts (not just diagnosed concussions) during youth and later-life neuropsychiatric and cognitive symptoms. The study used the

Cumulative Head Impact Index (CHII) as a way to quantify cumulative repetitive head impact (RHI) exposure from football; the CHII was calculated for all 93 participants.

The CHII index quantifies cumulative exposure across youth, high school, and college levels (Table 3):

		+				
Youth	% games played at second position	X	Position's weighted average no. impacts per season	X	Total no. of youth seasons	= |A|
		+				
	% games played at third position	X	Position's weighted average no. impacts per season	X	Total no. of youth seasons	
		+				
	% games played at first position	X	Position's weighted average # impacts per season	X	Total no. of youth seasons	
		+				
High school	% games played at second position	X	Position's weighted average no. impacts per season	X	Total no. of youth seasons	= |B|
		+				
	% games played at third position	X	Position's weighted average no. impacts per season	X	Total no. of youth seasons	
		+				
	% games played at first position	X	Position's weighted average no. impacts per season	X	Total no. of youth seasons	
		+				
College	% games played at second position	X	Position's weighted average no. impacts per season	X	Total no. of youth seasons	= |C|
		+				
	% games played at third position	X	Position's weighted average no. impacts per season	X	Total no. of youth seasons	
Cumulative Head Impacts Index = [A] + [B] + [C]						

Table 3: The formulas indicate the total sum of the position-weighted average impact counts multiplied by the percentage of games played by a certain position and overall seasons played across Youth (A), High School (B) and College (C).

The study found a positive relationship between the two variables: as CHII exposure increased, the risk of cognitive impairment in the subjects increased as well. The model found CHII thresholds above which risk started to significantly increase, indicating that the relationship was not linear from zero. This finding supports a dose-dependent relationship with repeat head impacts (including sub-concussive hits, not just concussions) and diminished long-term brain health, meaning the greater the number or intensity of blows to the head, the more severe the damage or the effect on the brain.

(Figure 1) below from the study displays the probability of developing one of the six measured variables (behavioral dysregulation, meta-cognitive impairment, executive function, depression, apathy, cognitive impairment) on the Y-axis, compared to the calculated CHII index on the X-axis.

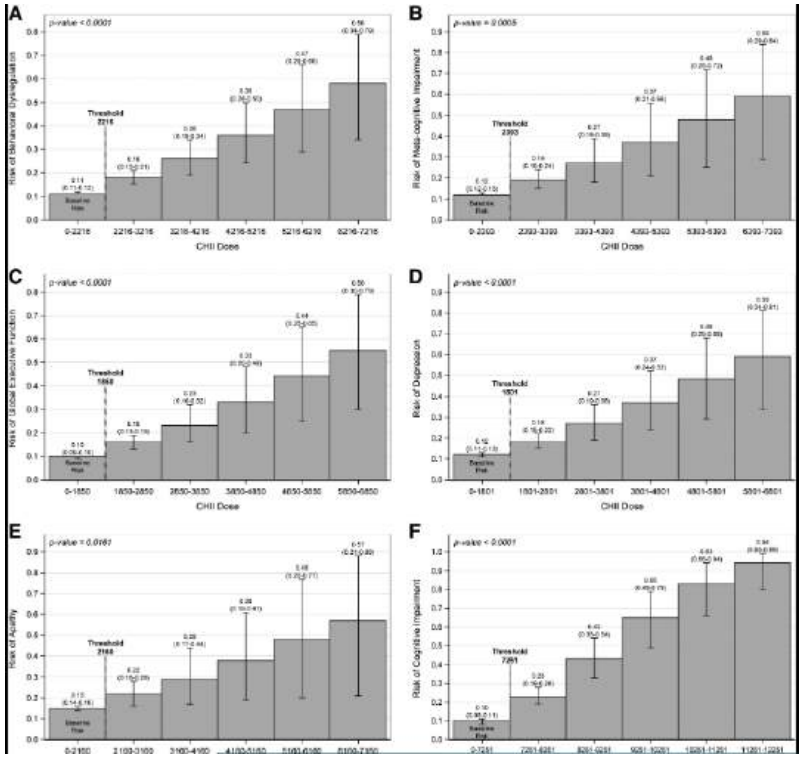


Figure 1: The figure from Montenigro et al., 2017, shows the probability of developing certain clinical impairments in relation to Cumulative Head Impact Index (CHII) exposure. The graphs show the probability of developing certain impairments, including: (A) Behavioral Dysregulation, (B) Meta-Cognitive Impairment, (C) Global Executive Function, (D) Depression, (E) Apathy, and (F) Cognitive Impairment. The dotted lines mark the point at which the probability of each clinical risk rises significantly ($p < 0.05$).

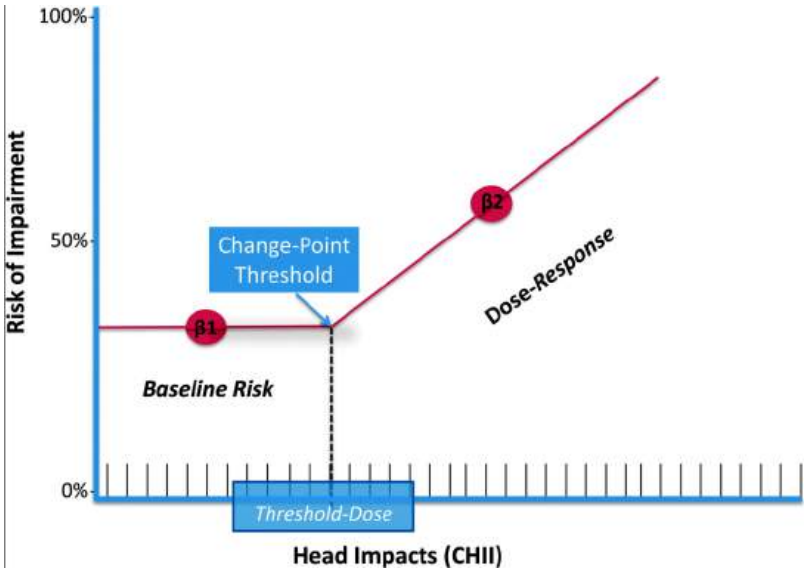


Figure 2 (above): The figure shows the two segments of the relationship between CHII and the risk of impairment. Risk is at baseline (B1) until cumulative exposures cross the change-point threshold. After the threshold is crossed, the risk increases through a positive dose-response relationship (B2).

In all six measured impairments (behavioral dysregulation, meta-cognitive impairment, executive function, depression, apathy, cognitive impairment), risk considerably increased once a particular threshold was reached (Figure 2). The most obvious example was cognitive impairment, which increased from an initial 10% (at a level of CHII exposure below 7,251) to 94% (at the topmost CHII exposure band of 11,251 to 12,251; $p < 0.0001$). Similar threshold effects were seen with depression (12% to 59%; threshold 1,801; $p < 0.0001$) and behavioral dysregulation (11% to 58%; threshold 2,216; $p < 0.0001$), which confirmed that the relationship between cumulative head injuries and clinical impairment outcomes is non-linear and rises significantly once a certain threshold is reached.

C. Behavioral and physiological Correlations

Behavioral and physiological symptoms are major indicators of CTE. These symptoms vary depending on what stage the disease has progressed to (ranging from Stages I-IV). The symptoms present in early stages of CTE (Stages I and II) include: headache and loss of attention and concentration for Stage I; depression, explosivity, and short-term memory loss, additionally for Stage II (McKee et al., 2013).

The symptoms present in the latter stages of CTE (Stages III and IV) include: executive dysfunction and cognitive impairment for Stage III; dementia, word-finding difficulty, and aggression for Stage IV (McKee et al., 2013).

These behavioral and physiological symptoms change as the patient goes through different stages of CTE. The early stages are more mood and behavioral issues like depression and aggression, while later stages are more marked by cognitive decline. (McKee et al., 2013).

3.5. Ethical, Social, and Policy Implications

A. Youth Participation in Contact Sports

The controversy around youth participation in contact sports is characterized by two sides to the argument: the social and physical benefits of youth sports and the dangers of the neurological damage that can arise from exposure to repetitive head injuries (RHI) at an early age.

In the United States, there have already been changes to youth concussion regulation. After Washington's Zackery Lystedt Law was passed in 2009, as a reaction to a brain injury of a 13-year-old athlete who had been playing while being unaware of his concussion, all 50 states and the District of Columbia adopted similar regulations by 2014 (Yang et al., 2017). The common goal of these laws is to establish concussion training for parents, coaches, and players, mandatory removal of a player from the game if a concussion is suspected, and require written permission from medical personnel to return to the game. The issue is that none of the existing legislation considers subconcussive injury and cumulative impact, as only a few states require baseline cognitive testing, and there are few requirements for doing any other preventive precautions recommended in this review. The actions described below are ways to improve

existing concussion regulations by monitoring long-term cumulative danger, which is currently not mentioned in existing laws.

Even though participation in any sport or activity is ultimately decided by the child's parents, these youth sport organizations have to ensure that there are protective policies in place for the young athletes, especially at such a critical time of development, to ensure the safety of athletes and help prevent trauma-induced neurodegenerative diseases.

B. Rule and Equipment Changes

It is important for youth sports leagues to establish rules regarding practice activities and equipment to protect their athletes. For practice standards, youth football leagues could set strict restrictions on the amount of time that players spend training in contact each week and outlaw dangerous head-on contact drills, such as the Oklahoma Drill, which unnecessarily exposes players to high-speed collisions.

Equipment also has to be further redesigned for safety. Guardian Caps, padded helmets that are designed to absorb impact energy, are an example of this type of preventive technology. The NFL introduced mandatory use of Guardian Caps for certain positions (such as linemen, tight ends, and linebackers) in practice in 2022, before extending it league-wide; the league reported a 54% decrease in concussion cases among these positions since the introduction of this mandate (Funk et al., 2025). The use of the caps has also gone beyond the professional level, with approximately 300 colleges, 3,000 high schools, and 750 youth programs using them (Hansen, 2024). Still, the data is slightly controversial: a study conducted by Hammer et al. (2025) tracked sports-related concussions (SRCs) for 2,610 high school football players over a football season. Athletes were classified as 'GC' if they wore a helmet and guardian cap during practice or 'Non-GC' if they only wore a helmet. Of 54 SRCs sustained during practice, 51.6% occurred in GC athletes and 31 (48.4%) in Non-GC athletes, meaning the athletes equipped with guardian caps composed a high proportion of all practice-sustained SRCs from the study. This can be explained by the potential risk compensation: the idea that players adopt riskier playing behavior when they feel safer because of the equipment. This still indicates that this type of protective equipment itself is not a complete solution and emphasizes the need for monitoring-based strategies such as accelerometers and biomarker screening.

One potential monitoring-based prevention strategy would be to attach linear and rotational accelerometers to the players' helmets and pads, which would provide sideline medical staff with immediate data on collision forces and speed, significantly decreasing the time between collisions and medical/support actions taken. The sensors would have a threshold of 60 -120gs of acceleration (the likely threshold range for concussion to occur). When an impact is measured at this velocity, medical staff on the sidelines will be alerted, and the players will immediately be taken into their care for clinical evaluation. For example, a study conducted by Eckner et al. (2011) provided 95 athletes from the same Class 3A football team with helmets equipped with a HITS encoder system. The encoder system contained six single-axis accelerometers, a battery, a data logger, and a wireless telemetry unit, and can be installed into

existing helmet cushioning without violating NOCSAE safety regulations or helmet performance. The monitoring technology records impact data during games and practices and sends this information in real time to a computer on the sideline. The athletes reported an overall mean value of 0.3 concussions during the season, a significant decrease from the 1.1 concussions on average by the athletes in prior seasons. This data proves that this type of monitoring with accelerometers is effective in preventing concussions over the season.

C. Screening and Prevention

A critical tool used to screen for potential side effects of head trauma would be routine neurocognitive testing of players. Tests like ImPACT and C3Logix measure memory, processing speed, and reaction time (Covassin et al., 2009). A baseline score would be taken by the athletes before the season; each athlete would then retake the test each week. If scores decline significantly from week to week, action will be taken, and the player will immediately enter a concussion-like protocol. Very few states currently require baseline cognitive testing in their youth concussion protocols, and none require biomarker or CHII-based monitoring.

Alongside the use of neurocognitive measures, the analysis of biomarkers from athletes' blood samples during the competitive season provides an opportunity to view the athletes' axonal health. Blood samples could be drawn from the athletes on a monthly basis. After receiving the samples, labs would then determine the concentration of NfL, p-Tau, GFAP, and UCH-L1 in the blood, which serve as indicators of neuronal damage. Routinely checking an athlete's blood sample is a clear way to determine and measure neurological damage in athletes by comparing the concentration of these biomarkers in tested athletes to the biomarker concentrations of clinically diagnosed CTE patients. While these biomarkers may serve as a sign of neurological damage in general rather than a distinct diagnosis of a certain neurological disease, elevated levels of a specific biomarker can act as an early warning system, allowing reduced contact or increased supervision of players to prevent permanent damage.

4. Conclusion

This review has explored existing research linking repeated head trauma in high school and college football players to early changes in brain structure, function, and chemistry. Findings include white matter loss after only one season of competitive football, the presence of CTE-related structural changes, including volume loss of the hippocampus and cavum septum pellucidum, and impacts resulting in the increase of UCH-L1 and tau biomarker levels by 740% and 500%, respectively. Concerning the statistical data, the work by Mez et al. (2017) showed that 21% of former high school football players and 91% of college football players had the signs of CTE identified in their bodies during the post-mortem analysis, while Montenigro et al. (2017) claimed that cognitive impairment rates rise from 10% to 94% with the increase of cumulative head injuries. This evidence has shown that the routine hits that athletes experience, which don't show any symptoms (sub-concussive hits), are the ones that have caused such large cognitive

degeneration, challenging the belief that only concussions could cause brain damage in athletes and drawing more attention to the accumulation of hits over seasons of play.

This review is limited by a number of constraints. As it was carried out as a narrative review and not as a systematic study, there are likely to be issues with selection bias since the sources were selected based on relevant factors and not through the use of any kind of pre-registered search protocol. A number of the cited studies are also based on small samples (e.g., the biomarker study involved 11 athletes and the DTI imaging study relied on 17 participants), as well as on convenience or post-mortem samples.

The ultimate goal is to implement a way to screen for brain damage in young athletes and prevent any possible signs of neurodegenerative conditions from developing even further. This can include: routine neurocognitive testing of players compared to baseline tests, MRI/blood tests to search for CTE/brain trauma biomarkers, calculation of the CHII index in athletes and evaluation of their risk of cognitive impairment from this number, and increased helmet technology, such as acceleration sensors to measure the degree of impact on the field.

As technology advances further, the risk of head trauma is likely to be reduced in the future, but it will never go away permanently. What we must do is find a balance between enjoying the benefits of sports and evaluating the risk that comes with them. Adapting preventive measures, such as those mentioned in this article, is the best way to reduce long-term effects of impacts to the head and preserve young athletes' brain health long into the future.

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Table and Figure Legends

Tables

Table 1. Cognitive and Behavioral Symptoms of Each Stage of CTE

Note. Adapted from McKee (2012). The table shows the different and specific cognitive and behavioral symptoms for each stage of CTE.

Table 2. Predictive Performance of Acceleration and Duration Metrics for CTE Diagnosis

Note. Adapted from Daneshvar et al. (2023). The table shows the accuracy of models in predicting CTE, comparing concussion count, player position, duration of play, CHII per 1,000 hits, cumulative linear acceleration (CHII-G), and cumulative rotational acceleration (CHII-R).

Table 3. Cumulative Head Impact Index (CHII) Calculation Formulas by Playing Level

Note. The formulas show the formulas used to calculate the CHII index: the sum of average impacts per position, multiplied by the percentage of games played per position and the total seasons played across the Youth (A), High School (B), and College (C) levels.

Figures

Figure 1. Risk of Six Impairment Domains Relative to Cumulative Head Impact Index (CHII) Exposure

Note. Adapted from Montenigro et al. (2017) . The figure shows the probability of developing six different clinical impairments: (A) Behavioral Dysregulation, (B) Meta-Cognitive Impairment, (C) Global Executive Function, (D) Depression, (E) Apathy, and (F) Cognitive Impairment. The dotted lines represent thresholds above which the probability increases significantly ($p < 0.05$).

Figure 2. Change-Point Threshold and Non-Linear Dose-Response

Note. The diagram shows the relationship between cumulative head impacts (CHI) and the probability of developing different clinical impairments. Once cumulative exposure reaches the threshold, the risk of developing the impairment increases in a positive dose-response relationship.

How does Chronic Stress (Measured by Self-Reported Methods) Affect Hippocampal Volume (Measured by MRI Scans) in Individuals Between the Ages of 9 to 20?
By Akalya Venkatesan

Introduction

Stress is the feeling of emotional or physical tension. Chronic stress is the prolonged feeling of stress and it happens due to the body being repeatedly exposed to stressful situations (Srakocic). In the body there is a complicated communication system that controls the body's reaction towards stress and it is known as the hypothalamus-pituitary-adrenal (HPA) axis (Cleveland Clinic). It begins with a cluster of neurones in the hypothalamus. A neurotransmitter called glutamate is released by the glutamatergic neurones in response to the stressful event and it diffuses across the synapse and binds to the receptors on parvocellular neurones causing corticotropin-releasing hormone (CRH) to be released (JST).The CRH then travels down the hypophyseal portal system (which is a network of blood vessels connecting the hypothalamus and the pituitary gland) and binds to G protein-coupled receptors (GPCRs) in the pituitary gland (Cleveland Clinic). This causes the activation of the G-protein which then activates an enzyme called adenylyl cyclase to release cAMP as a secondary messenger triggering a signal transduction. This results in the pituitary gland to synthesize and secrete adrenocorticotropic hormone (ACTH) (India et al.). This ACTH now acts as a ligand and moves to the adrenal glands (which are located above the kidneys) by travelling down the bloodstream and binding with melanocortin 2 receptor (MC2R) which are a type of GPCR (Knezevic et al.) (Angelousi). This triggers another signal transduction to release cAMP as a secondary messenger. The cAMP then activates protein kinase A which is an enzyme inside the adrenal cortex cells which triggers another enzyme to release the hormone cortisol (Romeo). This is how the feeling of stress is regulated (Mbiydzenyuy and Qulu). In chronic stress the stressful situation constantly activates the hypothalamus- pituitary-adrenal (HPA) axis causing a continuous release of cortisol (Cleveland Clinic).

In the temporal lobe of the brain, there is a S-shaped structure called the hippocampus. There are 2 of them in the brain, one on the right side and the other on the left side and they are each around 5 centimeters in length (Cleveland Clinic). The structures within the hippocampus include the dentate gyrus which processes new memories and help makes decisions, the cornu ammonis which consists of 4 regions (CA 1-4) which stores memories, the subiculum which collects signals from your brain and then uses the information to retrieve memories and the entorhinal cortex which is the pathway information travels from and to the Hippocampus (Cleveland Clinic). The hippocampus has 2 main functions: spatial memory (memories of places and directions) and episodic memory (memories of personal events) (Cleveland Clinic). It is extremely important for learning and decision making.

The process in which the brain forms new neurones is known as neurogenesis (Saaltink and Vreugdenhil). It takes place specifically in the dentate gyrus which is a part of the hippocampus, and is vital in memory formation. Another important process that aids memory

formation is called synaptic pruning, where the unused connections are removed while the important connections are strengthened. In synaptic pruning there are immune cells in the brain called microglia that engulf weaker synapses overall refining the brain and heightening neuroplasticity which is the brain's ability to adapt and respond to new experiences (Colonna and Butovsky)(Baker). However studies have shown that excessive levels of cortisol like seen when someone suffers from chronic stress inhibits neurogenesis (Blankenship et al.) and increases synaptic pruning (Frontiers in Behavioral Neuroscience) which affects the hippocampus.

Between the ages of 10-20 is often referred to as the adolescent period and chronic stress although unhealthy for all ages it is especially dangerous for adolescents. This is because during adolescent times the brain is still maturing,developing and forming connections hence they are the most susceptible to damage from chronic stress. Adolescent period is also the primary time for learning and a decrease in the volume of the hippocampus can cause memory impairment which would severely hinder learning ("Sleep Deprivation in Adolescent Mice").

The purpose of this investigation is to get a better understanding on how chronic stress affects the brain during adolescence in order to prevent any neurological issues that may occur to current and future adolescents. This topic is extremely significant as skills like decision making and the ability to learn and memorize information are needed the most during adolescence and it would be extremely detrimental if the shrinking of the hippocampus affected those skills. A survey taken in America has shown that the adolescent period is when people feel the most stress, having an average stress level of 5.8 on a scale out of 10. This is higher than the national average of 5.0 for adults. Furthermore 83% of adolescents specified that school as the main cause of stress and then worries about the future including university applications and career choosing being a close second (Change to Chill). In conclusion it is vital to fully understand the psychological and biological issues that come with chronic stress in order to make school a safer and better environment and improve overall mental health for current and future adolescents.

Methodology

For this investigation, 2 scientific articles: “Early Life Adversity Predicts Reduced Hippocampal Volume in the Adolescent Brain Cognitive Development Study” (Breslin et al., 2024, PMCID: PMC11264191) and a 2017 article with adolescent participants (PMCID: PMC5899620) will be analyzed. One reason these 2 journals will be used is that they are both from PubMed Central and are peer-reviewed articles meaning experts have looked over these journals hence their information has high credibility. Furthermore both journals follow strict yet well known methods which increases the credibility of the information.

In addition, the criteria for selecting the studies highly prioritized a strong relevance to the research question, a publish date of less than 10 years and the age of the participants and both studies were directly linked to the research question, were released 2017 and 2024 and the age of the participants in both studies were within the range of adolescents.

Results

The results from 2 research papers investigating the correlation between chronic stress and hippocampal volume will be stated and analyzed:

1. Early Life Adversity Predicts Reduced Hippocampal Volume in the Adolescent Brain Cognitive Development Study (Breslin et al 2024)

Early Life Adversity (ELA) score	Mean Hippocampal volume (mm^3)	Mean Hippocampal volume after 2 years (mm^3)
0	8220	8352
≥ 3	8039	8148

Table 1: Shows how the ELA scores compare the growth of the hippocampal volume over 2 years (Breslin et al.).

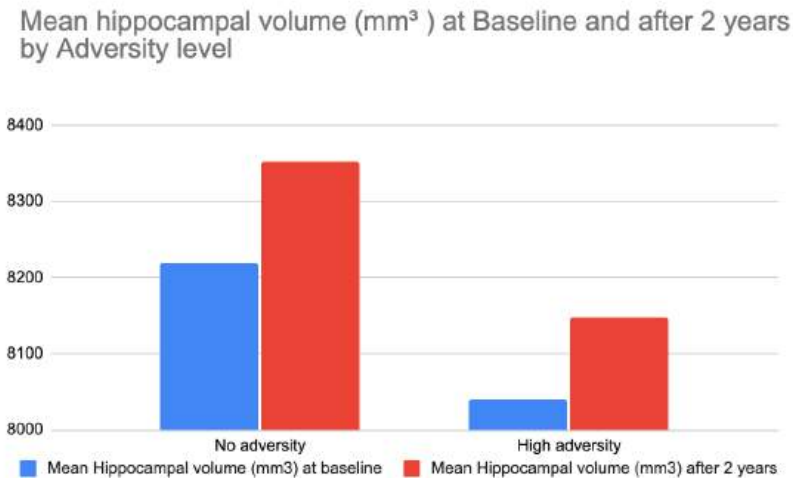


Fig 1: Compares the difference visually for the hippocampal volume at baseline and after 2 years for individuals with low and high adversity (Breslin et al.).

2. Perceived stress is associated with smaller hippocampal volume in adolescence (Piccolo et al 2019)

Hippocampal measure	Standardized β coefficient	p value
Total hippocampal volume	-0.17	0.017
Left hippocampal volume	-0.22	0.003

Table 2: Shows the standardised beta coefficient and p value when performing regression analysis on total hippocampal volume and left hippocampal volume. (Piccolo et al.)

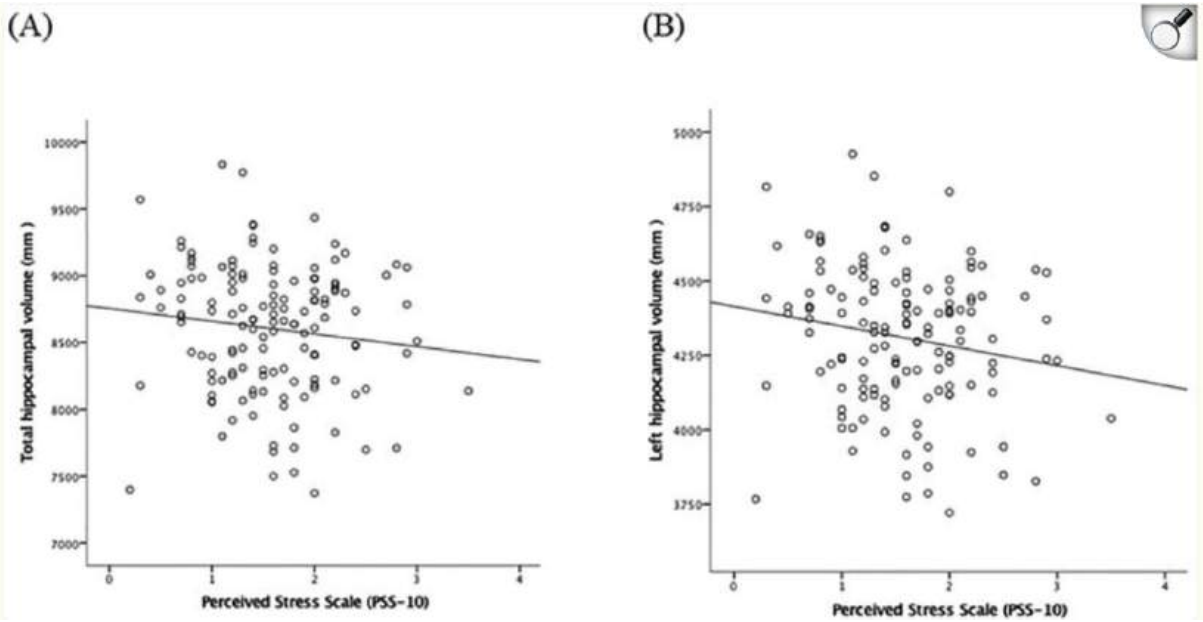


Fig 2: Scatter graph taken from Piccolo et al that shows there is a relationship between perceived stress scale (PSS-10) and hippocampal volume (Piccolo et al.)

Analysis

Both secondary studies show a negative correlation between stress level and hippocampal volume.

In table 1 and figure 4, it is seen that adolescents with higher early life adversity scores (ELA ≥ 3) had a smaller hippocampal volume (8039mm³) at baseline compared to children with no adversity (ELA score = 0) at baseline (8220mm³) (Breslin et al.). Over 2 years the hippocampal volume increased for both groups showing normal brain growth however adolescents exposed to no adversity had around 20% more growth than those exposed to higher adversity.

In study 2, stress was measured using a perceived stress scale (PSS-10) and the hippocampal volume was measured using an MRI. Table 2 shows the regression analysis of PSS-10 scores and the total hippocampal volume with standardized beta coefficient of -0.17 (Piccolo et al.) which shows a negative correlation between PSS-10 and total hippocampal volume. Furthermore the p value was 0.017 (Piccolo et al.) indicating that the results were statistically significant. It also shows that the effect of stress on hippocampal volume is more severe in the left hippocampus than the right hippocampus as the p value for the right hippocampus was >0.05 meaning the results were not statistically significant. Figure 5 is a scatter graph which further shows that there is a negative correlation between total/left hippocampal volume and PSS-10.

Overall, both studies show that higher chronic stress leads to smaller hippocampal volume and the biological reasoning for these results can be explained.

When someone suffers from chronic stress their HPA axis (the body`s communication system to regulate stress) is active for a long period of time leading to the adrenal glands releasing glucocorticoid hormones like cortisol. Usually under normal conditions, the glucocorticoids are released for a short period of time to cope with stress by increasing the body's alertness; however during chronic stress the glucocorticoids levels remain high for an extended period of time. The hippocampus is particularly vulnerable to chronic stress because it has a large amount of glucocorticoid receptors (GRs) causing the neurons in the hippocampus to be exposed to cortisol for a long time. This then causes glutamate, the brain's primary excitatory neurotransmitter released during everyday brain activity to aid learning and memory, levels to increase overstimulating excitatory receptors, specifically the NMDA receptors on neurons. The NMDA receptors are vital receptors that allow calcium ions (Ca 2+) to enter neurons for a brief amount of time in order to form memories, however an overstimulation of NMDA receptors can lead to excessive calcium influx into the neurons. (Popoli et al.)

Dendritic branches are extensions of neurons and are made of microtubules and actin filaments and they support synapses between 2 neurons. When the NMDA receptors are over activated, too much calcium enters the neurons leading to calcium activated enzymes such as proteases and phosphates to be activated. These enzymes then break down and destabilize proteins leading to the degustation of active filaments and the destabilizing of microtubules. Overall the cytoskeleton becomes weak. Furthermore, the dendrites require a lot of ATP in order to transport proteins along the microtubules, replace damaged cytoskeletal components and support synaptic activity; however, damages to the mitochondria reduce ATP production causing dendritic growth to reduce. (Popoli et al.)

The reason the left hippocampus was more severely affected than the right hippocampus was due to the left hippocampus being more involved in verbal memory and learning while the right hippocampus is more involved in spatial memory. With glutamate aiding memory and learning, higher levels of it affect the NMDA receptors on the left hippocampal neurons more than the right hippocampal neurons. (Ezzati et al.)

Brain-derived neurotrophic factor (BDNF) is a protein that promotes neurogenesis which is a process in which new neurons form. It does this by binding to a receptor on neurons called TrkB which promotes gene expression and protein synthesis. Usually the BDNF increases the number of neural stem cells in the dentate gyrus in order to assist the development of newer neurons and synapse formation however elevated glucocorticoids levels suppress the expression of the BDNF gene leading to decreased neurogenesis. Furthermore, a decrease in BDNF gene leads to a lack of growth and repair, decreasing the chances of surviving neurons and synaptic plasticity. (Hu et al.)

Due to all of this there is synaptic loss, reduced plasticity and neuronal death and overtime this can lead to reduced hippocampal volume.

Evaluation

Study 1 (Breslin et al 2024)

Study 1 investigated the relationship between early life adversity and hippocampal volume over 2 years. It included several thousands of participants aged between 9-12 and an early life adversity (ELA) score was calculated based on adverse experiences such as family issues, financial difficulties and unstable upbringing that the child experiences.

The results from study 1 has high validity as the sample size included 7036 children which is a large number meaning a large amount of data is able to be collected which minimizes the effect of participant variable on the results. It also increases statistical power and makes the results more generalizable and valid to individuals from different cultures and backgrounds. Furthermore, MRI scans were taken, once when the participant was 9-10 years old and then once again when the participant was 11-12 years old. This makes it a longitudinal study which is particularly beneficial for this topic as the brain grows over time and stress affects the brain structures over time so seeing development changes increases the accuracy of the results.

However it had some limitations, one being the ELA scores measure how many adverse experiences an individual has experienced rather than how much stress each one caused, especially since everyone responds to similar experiences differently this limits the validity of the results. Moreover, the information about the adversity was self-reported and collected from guardians and they may have either exaggerated or under-exaggerated the experience which may have affected the validity of the results. Lastly, since the amount of adversity an individual experiences cannot be manipulated due to ethical reasons it is hard to say that stress is the main and only cause for changes within the hippocampal volume for these individuals.

Study 2 (Piccolo et al 2019)

Study 2 investigated how stress is associated with smaller hippocampal volume in adolescence, it included 143 adolescents aged 12-20. It included participants filling out a questionnaire (PSS-10) and they got a stress score based on their answers. Then an MRI scan was taken in order to determine if there was a relationship between stress score and hippocampal volume.

The study had some strengths towards the validity of the results, one being they used the PSS-10 which is very well-known to be an accurate measure of how stressed an individual is. This increases the strength of the results as all participants answered this questionnaire meaning it was consistent. Secondly, they used the regression analysis, more specifically beta coefficient which allows the direction and strength of the relationship between stress and hippocampal volume to be quantified which can provide a stronger and more valid comparison.

However one limitation that did affect the validity of the results is the small sample size. Study 2 had 143 participants which is quite small especially compared to study 1 which has 7036 meaning the results from study 2 are less generalizable. Furthermore, the age range of participants was 8 years which is quite a large range and any brain changes or developments could have been caused by other reasons such as puberty or social experiences rather than age as individuals between those ages have the opportunity to experience a lot more.

Discussion

Although both studies show a negative correlation between chronic stress and hippocampal volume the studies don't prove that chronic stress is the direct cause of the reduced hippocampal growth. One alternative interpretation could be that a smaller hippocampus leads to chronic stress. As previously stated the hippocampus regulates the stress response through negative feedback of the HPA-axis where the hippocampus detects the rising cortisol levels and the hypothalamus signals it to reduce further cortisol release. However if the hippocampus is smaller it may be less functional and the negative feedback effect may be less efficient in regulating cortisol levels leading to higher cortisol levels and chronic stress rather than chronic stress leads to hippocampal volume.

Further research should prioritize more studies using a longitudinal design with repeated measures of stress and more MRI scans. Adolescence is a prime time for brain development and measuring the volume of the hippocampus just once or twice may not provide enough data about the correlation between chronic stress and hippocampal volume. Furthermore, this would allow us to determine whether reduced hippocampal growth happens before or after an increase in stress levels fixes the issue found in the current studies.

Moreover, in both secondary resources a self-reported method is used to collect information and determine an individual stress level; however self-reported methods can lead to someone either over or under-reporting their stress level. In order to be more accurate, future research should focus on using a biomarker for stress such as salivary cortisol that can be used to reduce bias and have a biological measure as well. Cortisol circulates the blood in 2 forms: bound cortisol which is when the cortisol binds to cortisol binding globulin and it is how the majority of the cortisol circulates the blood, and there is free cortisol which is small and non-polar and can diffuse down the concentration gradient from the blood to the saliva by diffusion. Including swab tests which can measure cortisol levels in saliva would allow researchers to directly link cortisol exposure to hippocampal development.

Further research could also investigate the correlation between BDNF levels as BDNF promotes neurogenesis and synaptic plasticity. Research on BDNF levels and hippocampal volume can determine whether a reduction in hippocampal volume is due to decreased neurogenesis and dendritic growth.

As previously mentioned adolescence is a prime time for hippocampal growth due to neurogenesis and synaptic pruning being extremely active. This could mean some recovery of reduced hippocampal volume may be achievable and this could be investigated through intervention studies which are types of studies where researchers apply a treatment, drug or activity on participants to measure its effect on health outcomes. For this topic, intervention studies can investigate the effect of exercise and improved sleep on cortisol levels and BDNF expression. If these studies show that improvement in hippocampal development is possible it can be implemented in hospitals and schools to decrease the chance of long term consequences discussed in this research paper from occurring.

Conclusion

This study investigated the following research question: How does Chronic Stress (measured by self-reported methods) affect Hippocampal Volume (measured by MRI scans) in Individuals between the Ages of 9 to 20?". From the evidence provided from both studies it shows those with chronic stress do have a reduced hippocampal growth leading to a smaller hippocampal volume.

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The Hidden Dangers of Concussions: Why Doctors Need Better Training in Diagnosis and Treatment By Ally Jacobsen

Every year millions of people — approximately 1.8 - 3.6 million—suffer the symptoms and lasting medical impairments from concussions. This group of affected individuals contains all ages and backgrounds - including children, high school athletes, professional athletes, car crash victims, and the elderly. The Mayo Clinic defines a concussion as a“mild traumatic brain injury that affects brain function” (Post-Concussion Syndrome”). Concussions are most often a result of a hard blow to the head that causes the brain to hit the skull, ultimately leaving the brain tissue harmed. Despite a variety of age groups being affected, many people within the general public remain under false pretenses that concussions heal quickly. However, emerging research reveals that concussions lead to serious long term problems like cognitive impairments, neurological disease, and emotional disorders. This is why doctors must receive more specialized training to properly diagnose and treat patients in order to prevent lasting damage.

All concussions, including those that are improperly treated, misdiagnosed, undiagnosed, and repeated are catalysts for serious and lasting damage to the brain and nervous system. Although the word “mild” is often used to describe traumatic brain injuries, such as concussions, this label is detrimental to people who suffer from them. The word “mild” hints at the injury being less serious, which misleads the general public and causes the severity of the injury to be overlooked. Also, medical experts emphasize that this misleading classification refers only to the initial visible structural damage seen in tests and scans, not the long-term neurological consequences that develop from the injury. Neurologist Charles H. Tator notes that “concussion is the most common type of mild traumatic brain injury and can have serious consequences” (Tator). When concussions go untreated or improperly monitored, they result in long term damage to the brain and nervous system, which are crucial for managing all bodily functions from the voluntary to involuntary actions like heart rate and digestion.

Understanding that there are many ways a concussion can occur is vital when speaking about traumatic brain injuries. Thus, medical professionals have developed a system to sort concussions into levels of severity. According to Broadview Health Centre, “the severity of a concussion is categorized using a grading system, denoted as Grade 1, Grade 2, or Grade 3 concussions” (“Understanding Concussion Grades”). This classification scheme elucidates that rather than being a single, consistent and uniform injury, concussions exist in the population on a spectrum of severity. Medical experts stress that concussion injuries are very specific and are highly individualized to each patient they affect. As the Broadview Health Centre article explains, “no two concussions are the same” (“Understanding Concussion Grades”), which sheds light onto how there is such a wide range of symptoms seen in patients after an injury to the head and how every concussion affects the brain in a unique way. Additionally, even amongst patients with the same grade of concussion, symptoms and long-term outcomes can differ greatly. Such variability is the reason why physicians can not diagnose and treat patients with concussions relying solely on a universal approach. Following a generic rubric is what causes medical

professionals to look over warning signs or underestimate the severity of an injury. Therefore, in order to guarantee that physicians can accurately assess each case and provide the individualized care necessary to prevent long-term neurological damage, improved education and concussion-specific training is vital and necessary.

Even mild, or Grade 1, concussions cause severe neurological symptoms that compound over time. It is said that Grade 1 concussions are the least severe because “in a Grade 1 concussion, there is no loss of consciousness" (“Understanding Concussion Grades”). Nonetheless, the lack of loss of consciousness does not correlate to the fact there was no damage to the brain and its neurological pathways; rather, any blow to the head has the capability to cause damage, whether or not the victim has lost consciousness. People with grade 1 concussions continue to experience headaches, dizziness, nausea and confusion after the injury (“Understanding Concussion Grades”). Cognitive issues like insomnia, light and sound sensitivity, exhaustion, short term memory loss, and difficulty concentrating on tasks often coexist with these physical symptoms. Individuals with traumatic brain injuries are more likely to suffer from memory issues, particularly when acquiring and or learning new information according to MSKTC (O’Neil-Pirozzi, Therese M., et al). Additionally, researchers note that short term memory is usually more affected than long term memory after a concussion (O’Neil-Pirozzi, Therese M., et al). Because short term memory is responsible for recalling recent information, these memory problems can impact everyday tasks like schoolwork, conversations, and decision-making because short term memory is in charge of recalling recent information. Furthermore, people with brain injuries might have trouble recalling upcoming tasks. Individuals who have suffered traumatic brain injuries, like concussions, may have trouble “remembering to do things in the future, such as keeping appointments or taking medications” according to the MSKTC (O’Neil-Pirozzi, Therese M., et al). These symptoms show how even mild concussions can significantly disrupt normal brain function and daily life.

Neurological damage can worsen if concussions happen frequently or are not treated adequately. Frequent head injuries have been linked to neurological degeneration and long term cognitive decline, according to studies. Repeated concussions may result in “delayed brain degeneration that leads to memory loss and movement disorders similar to Alzheimer’s and Parkinson’s diseases” (Tator). This data shows that neurological alterations resulting from concussion injuries can persist and develop long after the initial injury. The link between repeated concussions and a deterioration in brain function is further supported by additional research. A 2023 Oxford University study examining the long-term effects of concussion history found that “three or more concussions were associated with worse brain function in later life.” Researchers also found that individuals with multiple concussions performed worse on memory and thinking tests compared to individuals with no history of concussion (“Three or More”). These findings suggest that brain injuries caused by concussions can accumulate over time and gradually impair cognitive abilities such as memory, attention, and reasoning. The Oxford researchers also emphasized that “even a single concussion may have an impact on the brain,” demonstrating that concussion injuries should be taken seriously even when they occur only

once. Because the brain controls essential cognitive functions, repeated injuries can significantly increase the risk of long-term neurological problems.

Concussions can also lead to serious emotional and psychological problems because the brain regulates mood and behavior. Concussions cause persistent symptoms that can continue for long periods of time and affect many aspects of daily life. The Mayo Clinic states that these lingering symptoms that follow a concussion injury can persist for weeks, months, or even longer than a year, ultimately leaving a lasting impact on a person's ability to function normally . Additionally, the Mayo Clinic explains that people with persistent postconcussion symptoms often experience anxiety, depression, irritability and sleep disturbances ("Post-Concussion Syndrome"). These emotional symptoms happen because concussion injuries disrupt normal brain activity in areas that regulate and control our mood. Henderson further explains that concussions can affect cognitive and communication abilities such as memory, organization, attention, and speech, and that individuals with concussion-related brain injuries may struggle because "they may not be able to organize their thoughts: they may have a hard time processing new information; or they may have trouble finding the 'right' words" . These cognitive impairments highlight the impact that concussions have with everyday communication and decision making.

In addition to concussions causing impairments both emotionally and cognitively, traumatic brain injuries also disrupt the autonomic nervous system, or the division of the peripheral nervous system that controls involuntary bodily functions such as heart rate and blood circulation. It has been observed that this body system is altered in individuals who have experienced one or multiple concussions. A condition which affects the autonomic nervous system, referred to as POTS (Postural Orthostatic Tachycardia Syndrome), is seen in a large number of concussion patients. According to medical research, POTS can be seen following a traumatic brain injury, particularly when individuals experience long periods of inactivity during recovery as advised by their physician ("What is POTS"). This condition occurs when the nervous system does not properly regulate blood vessel tightening, which normally helps maintain stable blood pressure when a person stands up . As a result, gravity causes blood to pool in the lower body, forcing the heart to beat faster in order to maintain blood circulation. Individuals with POTS may experience symptoms such as lightheadedness, fatigue, brain fog, headaches, digestive problems, insomnia and difficulty exercising ("What is POTS"). Doctors commonly prescribe rest to patients who are experiencing the consequences of concussion, so POTS throws off the body's recovery, and instead of helping the brain heal, the body develops these secondary syndromes. The vast assortment of symptoms goes to prove that concussion injuries can affect not only cognitive processes but also essential physiological systems that regulate the basic functions of the body.

Despite concussions being a growing topic on the world stage because of the influx in the number of treated cases, many medical professionals are still receiving bare minimum and limited specialized training in diagnosing and managing these injuries. Because concussions affect complex neurological systems rather than being a single visible injury with one outcome,

accurate diagnosis and treatment needs to be rooted in specialized medical knowledge. Neurologist Charles H. Tator explains that a concussion is a "complex pathophysiological process affecting the brain, induced by biochemical forces." This complexity makes concussions incredibly different to treat in comparison to other common injuries such as broken bones or lacerations, which have standardized treatment procedures that are practiced heavily in medical school. While physicians commonly receive extensive training for treating visible physical injuries, concussion management requires the understanding of subtle neurological symptoms such as memory loss, attention problems, and emotional changes (Hallock et al). Because the brain controls attention, reasoning, and emotional regulation, there are a multitude of ways a blow to the head can injure any of these processes, creating a a wide variety of symptoms that may be difficult for physicians to identify without specialized training (Hallock et al.). As a result, many healthcare providers are not given the adequate resources they need to be completely successful in recognizing or managing concussion symptoms effectively.

An example of something major that accounts for this gap in training is the limited concussion-specific education provided by medical schools to their students. While medical students learn general information about traumatic brain injuries, specialized training in concussion diagnosis and management is often surface level. Research examining concussion treatment guidelines demonstrates that medical care for concussions frequently relies on limited evidence or expert opinion rather than standardized clinical training (Leddy). Moreover, concussion care guidelines can take years to be fully adopted into everyday medical practice. According to Leddy, it may take "about 17 years for new research evidence to be adopted into routine medical practice." This delay in research being assimilated into practice resulted in many physicians using outdated concussion management strategies, despite new research emerging with more effective diagnostic tools and treatment approaches. When patients are not receiving the most updated research available, it can slow their path to recovery. Since concussion science and neuroscience as a whole is rapidly evolving, physicians must stay at the same pace and should receive continuing education to remain informed about the latest medical research and treatment methods.

The lack of standardized concussion education available for practitioners is the reason the current medical guidelines and treatment plans are so inconsistent when correctly evaluating a concussion. Despite the fact that doctors have instituted somewhat useful plans like the "return to play protocol" for athletes, significant gaps in standardized protocols still exist for many patient populations. For instance, research shows that while clinical practice guidelines exist for pediatric and sports-related concussions, there are fewer clear guidelines for adults who are treated in emergency departments and discharged without hospitalization (Leddy). In addition, there are also extensive issues with the "return to play protocol", one being that it focuses on athletes getting back in the game, and not healing the concussion. Rather than tracking all of the effects of concussion like wobbly balance and delayed reaction time, the protocol for athletes zones in on just headaches, overlooking the other damage. This is why repeated concussions happen, because these minute symptoms that are easy to overlook make the patient vulnerable

and susceptible for accidents to occur. Another reason current concussion treatment plans are less than successful is because they are often based on limited research, highlighting the need for continued physician education and improved medical training (Leddy). Because concussion research continues to evolve, physicians must stay updated on new diagnostic methods and treatment strategies. Without the standardized education and the continuation of medical training, many healthcare providers will not be fully informed about the latest advancements in concussion care.

To conclude, concussions are far more complex and dangerous than people assume, and the evidence clearly shows they can cause serious long-term neurological and psychological damage if they are not properly diagnosed and treated. Therefore, because these injuries affect the brain's complex neurological systems rather than producing a visible physical wound, physicians must receive improved and specialized training in concussion diagnosis and management. Furthermore, research throughout this paper has demonstrated how understanding the long-term effects of concussions, addressing gaps in current medical education, and implementing stronger concussion-focused training programs are essential steps toward improving patient care and preventing misdiagnosis. Expanding medical education in this area would also allow physicians to better recognize subtle neurological symptoms, apply updated treatment guidelines, and carefully monitor patients during recovery.

Furthermore, due in large part to increased awareness of professional athletes, football players, and Olympic competitors who have sustained repeated head injuries, concussions have become a topic of recent discovery in the country. However, the risk of concussion is not limited to the professional world; it also affects students, young athletes, accident victims, and regular people. Therefore, in order to safeguard the long term health of millions of people, it is essential to improve the quality of physician care that patients receive through education, since concussions can affect anyone, regardless of athletic status. In the end, improving medical professionals' concussion training will help lower avoidable brain injuries, enhance treatment results, and guarantee that everyone who is at risk gets the protection and care they require.

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Adjusting Traditional Crop Rotations to Improve Long-Term Soil and Crop Health

By Iris Ornek

Abstract

The farming industry is one of the most important industries for the human race. It provides us with the food we need to sustain ourselves. However, studies have found that many of the farming techniques used today deplete and erode the soil. Numerous studies have made it clear that no-tilling practices, as well as diversified farming, are healthiest for the environment and better maintain farming practices in the long term. Numerous studies in the Midwest have also focused on studying the effects of existing systems as opposed to new or less common ones. However, there have been few controlled experiments that delve deeper into the topic of crop rotation and how different crops affect the soil. This study aims to take a closer look at a broad range of crops and how they change the soil composition over time. This study also aims to provide useful data for forming effective crop rotations in the region. Through this study, it has been made clear that nitrogen (N) and potassium (K) are much more variable than other nutrients. The data also provides a guide that can help farmers pick crop rotations that make sense for them while still keeping the soil healthy.

Keywords: Crop rotation, nitrogen (N) soil concentration, phosphorus (P) soil concentration, pH, potassium (K) soil concentration, soil health

Introduction

The farming industry is essential to the human race. Modern farming uses technological and scientific advances in the field, including pesticides, herbicides, fertilizers, machinery, and changes in farming techniques. Among many changes, researchers assert that tilling and monocropping hurt the soil and the environment. Despite numerous studies on the effect of tilling on crop yield and carbon sequestration, less is known about the effects of our crops on the soil. Crop rotations serve as a simple yet effective solution that many farmers use to protect their land and improve their yields. They are a farming technique that keeps the soil continuously covered with several different crops in order to reduce erosion and keep the soil intact. Oftentimes, cash crops are rotated with cover crops that remain on the soil during the off-season. This study investigates the effect of crops on the soil in order to determine a better option for crop rotations for Midwestern farmers.

Literature Review

Common midwestern crop rotation

In the Midwest, agriculture is dominated by monocropping and rotation cropping of corn and soybeans. These two crops have a significant place in the current agricultural industry and are in many everyday products. Researchers continue to do agricultural research; Wu, Davis, &

Sohngen found that soybean-corn crop rotations are necessary economically, but corn has a better impact on the soil (2025). Thus, it was recommended that corn be rotated more often. However, many farmers maintain equal or soybean-heavy crop rotations or monocrop corn and soybeans.

Many farmers utilize cover crops and other easy-to-maintain crops for the off-season, to prevent erosion and other negative environmental impacts (Balis et al., 2024). These cover crops are somewhat effective in preventing environmental issues as they protect the soil while the farmer is not using it. However, researchers still raise the question of whether other aspects of the system could be made better. Adding cover crops and seasonal crops may maintain soil fertility to an extent; however, many researchers identify tilling as a larger indicator of soil erosion. Thus, soil erosion becomes an underlying issue, with a lot of research dedicated to its reduction or even elimination.

Issues with the current agricultural system

Some of the most prominent issues in commercial farming stem from farmers' practices. Erosion, for example, is prominent in the Midwest, where tillage practices are widely used (Kwang, Thaler, & Larsen, 2023). The erosion from tilling ultimately removes/decreases the amount of soil organic carbon (SOC) in the soil (Kwang, Thaler, & Larsen, 2023). This decrease in SOC reduces the amount of nutrients, water, and other essential factors that are stored in the soil. Without a proper source of SOC, crops are unable to grow to their full potential, negatively impacting the farmer and consumer, in addition to its negative impacts on the environment. However, tilling has been widely researched and recognized as a practice that the farming community should transition away from due to its negative impact on the soil and environment.

Because tillage has a large influence on erosion, it is important to take into consideration the effect of switching crops often. One characteristic of today's crop rotation is that farmers till between crops to clear and reset the soil for planting. While crop rotations are important to the overall health and diversity of the soil, as well as the economic necessity for different crops, it is important to balance how many crops farmers switch into rotation. Research suggests that excessively rotating crops while maintaining tilling practices may have negative effects that begin to outweigh the positive ones; this must be taken into consideration. Thus, the potential to move to no-till or low-till processes may be a beneficial adjustment to make in addition to crop rotation.

Diversified and Organic Farming vs. Non-Diversified Farming

There is a comparable attitude between diversified and non-diversified farmers. Non-diversified farmers are mostly set in their ways. They are economically and resourcefully unchallenged and therefore find no value in changing for financial reasons (Traldi et al., 2024). Resources such as fertilizer and planting guides are more plentiful for non-diversified farmers because non-diversified farming is far more traditional and common. In turn, there is much more technology and information available for those crops, whereas diversified farmers may have less

abundant suppliers. This, of course, is a limitation to take into consideration when transitioning into diversified farming. The hope is that as diversified farming becomes more common and research becomes more prevalent, resources will also grow.

Diversified farmers have been able to farm to a similar capacity as non-diversified farmers because of increasingly better technology (in both size and performance) (Traldi et al., 2024). Without these technologies, the same level of revenue, yield, and quality would not be reached. The attitudes of farmers towards changing their traditional farming techniques are important to consider, as a solution to the environmental issues in agriculture must seem attainable– and worth it– to the farmer or company.

Relationships between soil contents, health, and crop choice

Research hasn't shown a clear standard for what the soil should contain. However, many different macronutrients and micronutrients have been identified, as well as other crop-influencing factors, such as pH and soil type (including density and consistency). The most prevalent macronutrients and micronutrients seen through various pieces of literature include: nitrogen (nitrate), phosphorus, soil organic matter (SOM), potassium, magnesium, and calcium. Nitrogen has been particularly associated with the use of fertilizers and its ability to increase nitrogenous content. Research has found that higher nitrogen concentrations can benefit crops such as corn and soybeans, but they also raise concerns about runoff and suggest a reduction in nitrogenous fertilizer use in fields. There have also been studies that find several correlations between different nutrients and their ability to improve crop yields. Generally, many of the nutrients have a positive correlation with crop yield. However, it is important to consider the optimal range of nutrients, as at some point the crops reach their maximum absorption rate. Finding this optimum range can help farmers better understand the amount of money they need to spend on soil-enhancing treatments, equipment, etc. It also ensures that nutrients that are not absorbed by the crops do not become runoff.

One particularly detailed study conducted by Drescher et al. found that corn yields had an optimum potassium (K) range of 110 ppm and a phosphorus (P) range of 36 ppm (2021). This optimal number was defined as 95% yield of corn and was further linked to its cost-effectiveness for farmers. This is because less potassium and phosphorus fertilizer was needed to make recognizable increases in yield. In fact, the researchers found that fertilizer was only needed to maintain the optimum levels at 95% yield (Drescher et al., 2021).

A similar concept of an optimal range of nutrients for tomatoes and cucumbers indicated that the optimal concentration of Magnesium (Mg) was 2 mol/L (Qu et al., 2023). Results showed that adding amounts of Mg over 2 mol/L decreased the biomass of the plants rather than maintaining or slightly increasing it (Qu et al., 2023). This diverges from Drescher et al.'s research, which found that increased nutrient fertilizers wouldn't necessarily hurt the soil (although excess fertilizer could cause runoff) but would have minimal positive effects on crop yield (2021). Regardless, both points together provide insight into the environmental and economic importance of keeping the soil in its optimal range. Over-enriching the soil may prove

to be more harmful to the environment at times, as it increases fertilizer runoff and decreases crop yield and size. Unnecessary enriching does not only have the potential to create runoff, but it also has the potential to increase costs.

Gap

Researchers have expressed various gaps within agricultural and soil sciences; they are all connected into one large gap, and this research project aims to fill a small part of that. One main gap that researchers among multiple papers expressed was the lack of foundational knowledge about nutrients within the soil. Martin et. al., express the importance of linking information about soil nutrients to crop yield and health (2022). Much of the research done was under uncontrolled conditions and was mostly situational to the farm, area, and conditions.

The main gap is that this paper focuses on crop rotations specifically; this is something that hasn't been extensively studied but could provide important recommendations that could help both the farmer and the environment (Wu, Davis, & Sohngen, 2025). This study's goal is to collect nutrient data that can help to harness changes in soil nutrients throughout the growing season to maximize yields while maintaining long-term soil integrity.

Given the time and resource constraints, it would be impossible to perform a perfect experiment that takes into account every variable and its effect on the experiment. However, even a smaller-scale experiment could begin the conversation about sustainable soil practices and could begin the creation of a small, yet useful, concept for agricultural use.

Methodology

Experimental Design

To determine how rotations can adapt to harness nutrient changes throughout the growing season, maximizing yields while maintaining long-term soil integrity, I performed a controlled experiment. Running an experiment on the soil allowed me to isolate certain variables to find relationships and trends without variations in environmental conditions. Many existing studies have used field studies and surveys to gather data about existing crop systems and soil health. This created an abundance of information about individual systems, but no relationship to or development of agricultural practices. Studies such as Wu, Davis, & Sohngen expressed that a lot of research must be done in the field and that controlled experiments that focus on more specific aspects of agriculture are beneficial for the field (2025). Part of the gap surrounding soil science and agricultural research is the lack of experimentation and data collection from controlled environments. Many researchers are trying to bridge economic and environmental research in order to form a better system for the future. Connecting the gap provides a better foundation for the argument that agriculture needs to adjust to the ever-changing environment.

Hypothesis

I hypothesized that different types of crops would remove and add certain nutrients through their root systems. This will vary from crop to crop because they have different nutrient necessities. Thus, keeping the soil covered and rotated will protect soil health and crop yield while reducing cost because the crops will nearly keep the necessary nutrient balance in the soil and prevent erosion.

My hypothesis lacked some certainty and specificity because the minimal research present on the topic does not present a variety of tested crop rotations. This experiment was unique from others, and therefore, interpreting the results of it proved useful for the field.

A null hypothesis was also established: the type of crop and/or soil does not impact nutrient loss and gain trends over 6 weeks. If the null hypothesis is accepted, it will mean that farmers should employ other methods to create the best crop rotation.

Materials

The materials of this experiment consisted of the following items (Table 1):

Category	**Materials**
Testing	- LaMotte Testing Kit - Tests for pH, K, P, and N - Measuring tape - Compartmented container (plastic)
Directly affected in the experiment (organic)	- Traditional potting soil - Sand - Water - Seeds (corn, soybean, red clover, alfalfa, cowpeas, switchgrass, and asparagus)
Setup	- Pots - Scale - Growing cart - Tape - Pen

Table 1: The left column shows the categories of the materials listed, while the right shows the specific materials used in the experiment. Testing refers to the materials used to measure the change in soil nutrients and qualitative health indicators over time. Organic materials directly affected in the experiment refer to organic materials that are being tested/required to keep the plants alive. Setup refers to standard materials in the experiment that do not interact with the soil.

Site Information

This experiment occurred at BEHS's greenhouse from December through February. The plants were grown under natural light in the greenhouse. The average daylight time was approximately 9 hours. The greenhouse temperature averaged 21℃, with a range of 27℃ - 16℃.

Procedure, Variables, and Setup

With over 30 variables to be tested and taken into account, this experiment sought to test a select few variables that have a known impact on crop yield and soil health, but have relatively little information regarding numerical trends and connections. The most identified variables in past research were pH, SOM, SOC, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg). Testing these variables helped to identify what certain plants need to survive, and in the past, has been used as a marker for soil health. However, due to resource constraints, I opted to test pH, nitrogen, phosphorus, and potassium as the markers for soil health in this experiment.

The experiment was set up to test two separate independent variables: type of soil and type of plant. As the plants grew, the changes in the markers for soil health (pH, K, P, N) were measured. In order to make sure the two independent variables did not influence each other, every trial of a type of plant was performed in the same soil. Additionally, those trials were replicated, only changing the type of soil (See Table 2 below). The trials were done over 6 weeks, in a two-story structure under natural light in the greenhouse. The plants that were tested include: Knuckle Purple Hull Cowpeas (*Vigna unguiculata*), Chiba Green Soybeans (*Glycine max*), Trucker's Favorite Yellow Dent Corn Seeds (*Zea mays)*, Red Clover (*Trifolium pratense*), Switchgrass (*Panicum virgatum*), Alfalfa (*Medicago sativa*), and Mary Washington Asparagus (*Asparagus officinalis*). For simplicity, potting soil was used for all trials, but half of the trials contained potting soil with added sand content (2:1, soil:sand). Over time, the soil was tested for the soil health markers previously mentioned.

To set up a controlled environment for the experiment, the plants were placed in the same area of the greenhouse where they could receive equal amounts of sunlight. The structure was 1.5 ft x 6 ft x 6 ft and placed near the door of the greenhouse, facing South. Plants were grown in separate pots as indicated in Table 2.

	Potting Soil			Soil w/High Sand Concentration		
Dent Corn	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Soybean	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Cowpeas	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Red Clover	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Switchgrass	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Asparagus	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Alfalfa	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3

Table 2: This table explains how the 42 trials are distributed among the two variables. This shows the type of soil and crop that are applied in each trial (3 of each).

A bucket of fresh potting soil was removed from the main bag and mixed with 5 scoops of sand to create the sand-based soil variable. The soil was already at the proper hydration level; however, if that was not the case, the soil would first be mixed with water until it reached a moist consistency to set a base hydration level. Each soil type was tested to find the initial nutrient levels. Soil was then added to the pots by weight.

Every pot received 2-3 seeds to account for the chance of unviable seeds. This excluded denser crops that don't need to be spaced out: red clover, alfalfa, and switchgrass. Instead, these crops received a thin layer of seeds at their necessary depths according to the instructions on the package. The plants were then placed in the greenhouse, marking the beginning of the experiment.

Soil testing occurred before the start of the trial period, once throughout Week 3, and at the end of the trial. To account for the time and labor that were required to precisely test soil samples, the collected soil samples were stored in compartments in order to allow for testing over several days.

Additionally, the soil was watered approximately every other day, watering by weight to ensure that the plants were neither underwatered nor overwatered. This method was developed by Purdue University, and followed their instructions.

Analysis and Data Collection

Quantitative analysis was done in a numbered system that correlated to levels none, trace, low, medium, high, and very high, which had corresponding ranges in lbs/acres (See Table 3.1 below).

Key of Amounts	Phosphorus	Potassium	Nitrogen
Trace	lower end of range	lower end of range	lower end of range
Low	0-50 lb/acre	0-120 lbs/acre	0-30 lb/acre
Medium	50-100 lb/acre	120-200 lb/acre	30-60 lbs/acre
High	>100 lb/acre	>200 lbs/acre	>60 lb/acre

Table 3.1 represents the range of lb/acre for each tag (trace, low, medium, high) and each nutrient.

pH was an exception to this system, where its raw values were recorded and graphed. Potassium was measured by the number of indicator drops used during testing. This was done to accommodate the unique testing method for measuring potassium concentrations. Additionally, when reading graphing data, a positive slope indicated a decline in potassium. The collection of data was mainly quantitative; some qualitative points were informally noted. These include perceived soil texture, perceived plant health (color, presence of wilting, etc), and observations such as water retention, root structure, height, and plant behavior.

Soil tests were collected, averaged, and plotted with time (weeks) on the x-axis. Two different visuals were made: one with nutrient concentrations on the y-axis, and the other with crops on the y-axis.

After all data was collected and plotted, a crop rotation plan was formulated by referencing the data. This answered the second part of the research question, which specifically inquires about the most efficient crop rotation for Midwest farmers.

Limitations

The two main limitations of this experiment were time constraints and funding/resource constraints. While I derived various important nutrients that could show significant changes, the available testing methods allowed me to only test for changes in pH, nitrogen, phosphorus, and potassium. Additionally, given the scale of this study, the nutrient testing was based on color indicators which caused the data to act only as ranges or rough estimates. Regardless, the tests did provide insight into the overall trend of nutrient concentration in the soil.

An in-depth analysis of the soil concentration, as well as frequent testing of all 42 tests, was laborious. To help combat the issue, soil samples were taken on the same day and stored, allowing for testing over several days. However, the number of tests was restricted to a pre-trial test, a 3-week test, and a final test in order to maximize time for the most accurate results.

There was a source of error related to general access to the greenhouse (as restricted by school hours) and human error. Most notably, I was unable to access the school or travel too much due to the extreme cold that closed the school. Although only left alone for about a week, the plants did suffer stress and wilting. This was due to a power outage, which dropped the temperature of the greenhouse; thus, the trial ended at Week 6.

Overall, while limitations do persist, they did not greatly affect the trends seen as a result of the experiment.

Results

Throughout the 6 weeks, the data recorded showed consistent values for pH and phosphorus among all crops. The variation, though slight, was most obvious in the data recorded for potassium and nitrogen. These trends also varied by crop. Therefore, the results seen for potassium and nitrogen should drive the construction of a crop rotation.

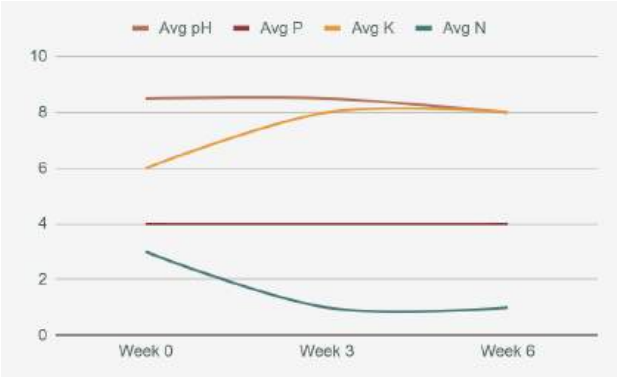
Sand-Based Soil v Potting Soil

The type of soil had little effect on changes in nutrient concentration. However, there were apparent differences in physical characteristics such as water retention and density. For example, there was a much higher retention of water in the sandy (sand-based) soils compared to the potting soils. This was most noticeable during weight checks when the potting soil had lost much more water weight than the sandy soils. Additionally, when boring holes to take soil samples, there was a clear difference in the density of the soil. The sandy soil moved easily, while the potting soils required much more effort to reach the bottom for testing. Pots containing

sand-based soils had slightly larger root structures than potting soil crops, most likely due to less dense soils that allowed for the roots to penetrate deeper.

Soybean

Soybean crops remained relatively neutral. The nitrogen and potassium concentrations decreased slightly over the 6-week trial: the change in potassium was not very significant, as it still read as "very high" on the color chart, whereas nitrogen concentrations dropped from medium (3) to trace (1) by Week 6 in the potting soil. The same trend for potassium concentrations was apparent in the sandy soil. However, nitrogen concentrations increased slightly over the 6-week trial from trace levels (1) to low levels (2). When testing for potassium in both soil types, the number of indicator drops increased from 6 drops (potting) and 7 drops (sand) to 8 drops. This indicated that potassium concentrations remained in the high range of over 200 lbs/acre, but had dropped slightly within that range throughout time.



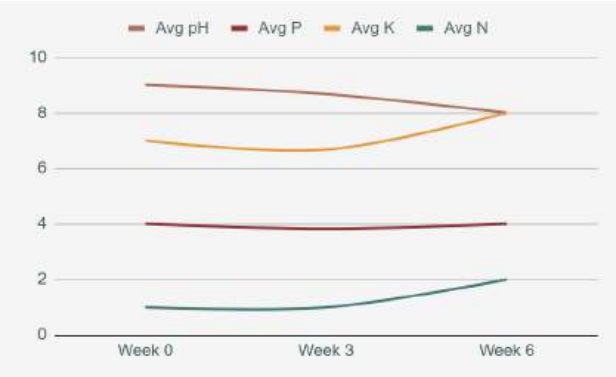
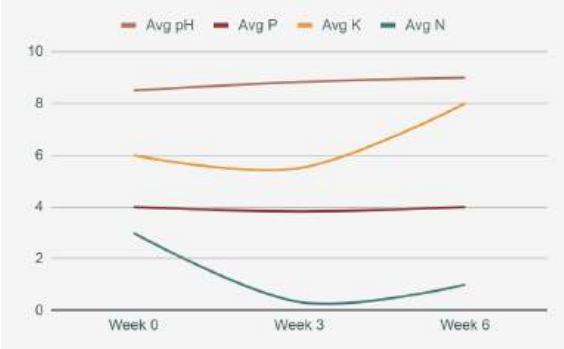


Figure 1.1 (left) & Figure 1.2 (right): Graphs of nutrient data for soybean pots. Figure 1.1 represents data collected from pots containing potting soil, while Figure 1.2 represents data collected from pots containing sand-based soil.

Figures 1.1 and 1.2 show slight differences among the soil types. Most notably, soybeans replenished the nitrogen concentrations in sand-based soil, while they used up more nitrogen in the potting soil trials. Additionally, the soybean plants were generally more successful in the sand-based soil, as all three trials grew quickly and strongly, while only one potting soil soybean plant successfully sprouted.

Red Clover

Similar to the soybeans, red clover's potassium concentrations decreased slightly but maintained a very high concentration. They also maintained even levels for pH and P, a commonality among all crops and soil types. The average nitrogen for potting soil plants decreased from a medium concentration (3) down to traces of nitrogen (1) by Week 6. The average potassium also saw a slight decrease in concentration, though, similar to the soybean, potassium concentrations remained very high. The sand-based soil crops had similar trends; however, nitrogen and potassium average concentrations showed some variation, but generally remained more stable than their potting soil counterparts.



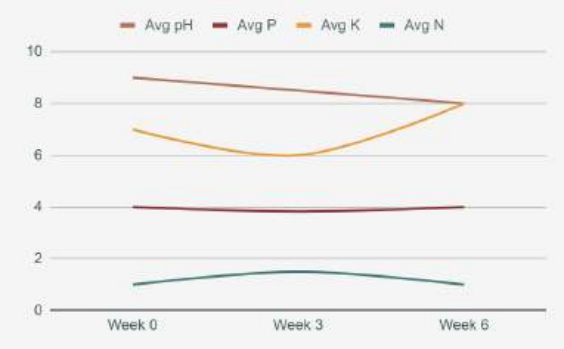
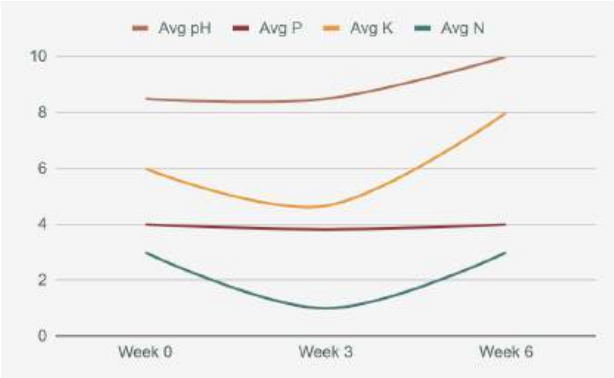


Figure 1.3 (left) & Figure 1.4 (right): Graphs of nutrient data for red clover pots. Figure 1.3 represents data collected from pots containing potting soil, while Figure 1.4 represents data collected from pots containing sand-based soil.

Figures 1.3 and 1.4 visualize the similarities and differences between soil types. The soil remained basic in both groups, with the pH consistently testing between 8 and 10. Additionally, despite both groups having different initial nitrogen concentrations, the red clover crops left traces of nitrogen (1) in the soil. Pot 4 (red clover, potting soil) was removed from the potassium data as it was an outlier in the data. This was most likely due to human error during the testing process. All of the red clover crops were green and healthy throughout all 6 weeks. They formed a dense, short layer of greenery that grew more quickly compared to other crops.

Dent Corn

Similar to red clover, Dent corn had consistent results among both soil groups, with the only variation being for nitrogen concentrations. pH in both groups increased slightly, becoming slightly more basic. Phosphorus remained constant throughout all 6 weeks. Interestingly, nitrogen levels were initially depleted at 3 weeks. However, 6-week results showed that average nitrogen concentrations had increased back to a medium concentration (3). This was particularly unique for the sand-based soil trials because the initial nitrogen concentration was a trace (1). There was also a slight increase in average potassium concentrations in the potting soil group at Week 3, followed by a slight drop by Week 6.



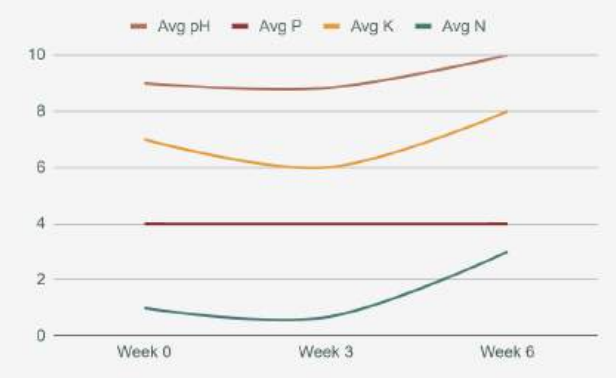
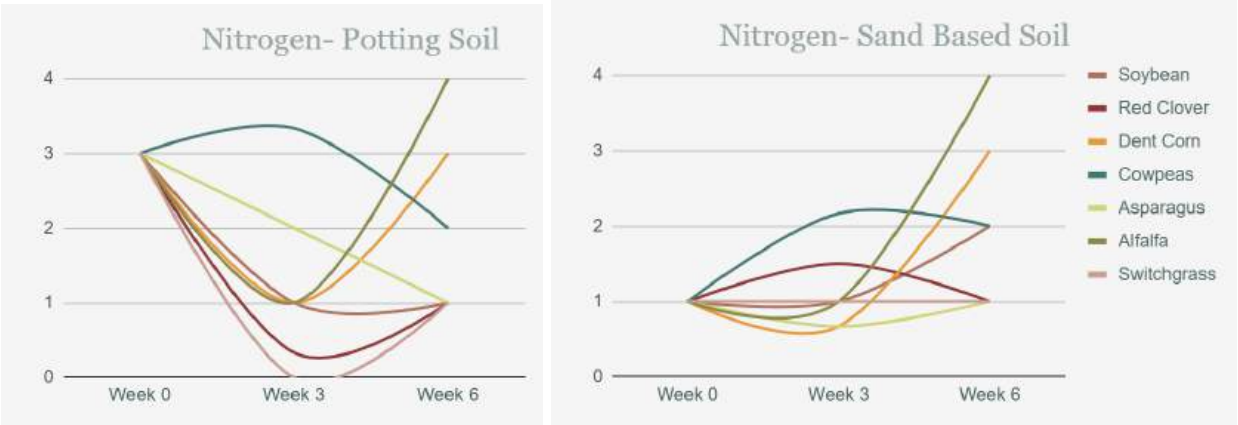


Figure 1.5 (left) & Figure 1.6 (right): Graphs of nutrient data for Dent corn pots. Figure 1.5 represents data collected from pots containing potting soil, while Figure 1.6 represents data collected from pots containing sand-based soil.

Figures 1.5 and 1.6 resemble those of the red clover graphs, with potassium slightly decreasing and nitrogen slightly increasing, but remaining low throughout the 6 weeks. The pH in both groups remained between 8 and 10, maintaining a basic soil. Phosphorus remained high throughout all four weeks with little variation.

Cowpeas

Cowpeas showed the most variation among all the crops. In particular, there was a clear increase in nitrogen concentration in the soil by Week 3 for both soil groups. However, by Week 6, the nitrogen concentrations for cowpeas had decreased slightly, reaching a similar concentration of around 0-30 lbs/acre or a low concentration (2). Other nutrients showed similar trends to the rest of the crops (See Figures 1.7 and 1.8 below).

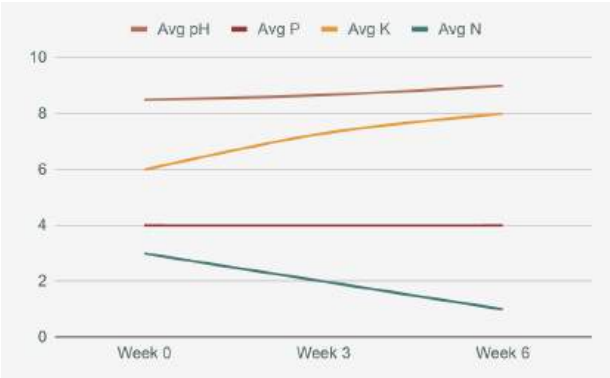


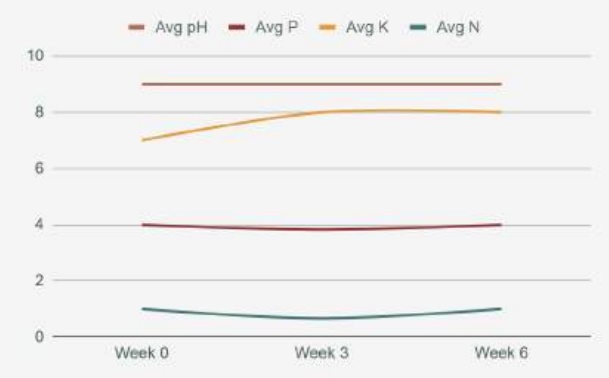
Figures 1.7 (left) & 1.8 (right) compare the change in nitrogen concentration for all crops; both potting soil and sand-based soil are depicted in separate figures.

Figure 1.7 and 1.8 clearly show the contrast between average nitrogen concentration trends for cowpeas. Cowpeas maintained a higher nitrogen concentration throughout all 6 weeks, despite having only traces of nitrogen in the sand-based soil. By Week 3, the potting soil crops had increased to a medium/high concentration, and the sand-based crops had increased to a low/medium concentration, after initial concentrations of medium and trace, respectively. By Week 6, both groups had slightly decreased to a low concentration of nitrogen (2).

Asparagus

Asparagus represented perennials in this experiment. Unlike many of the other crops, all nutrients in asparagus crops were constant for all 6 weeks. The only variation in average concentrations was in the potting soil group, where nitrogen and potassium dropped slightly as the crops absorbed nutrients. Regardless, the change in potassium remained in the “very-high” column of the testing kit, making the decrease very slight. Additionally, the change in nitrogen was a slightly more significant drop, decreasing from an average medium (3) concentration to a trace of nitrogen (1).

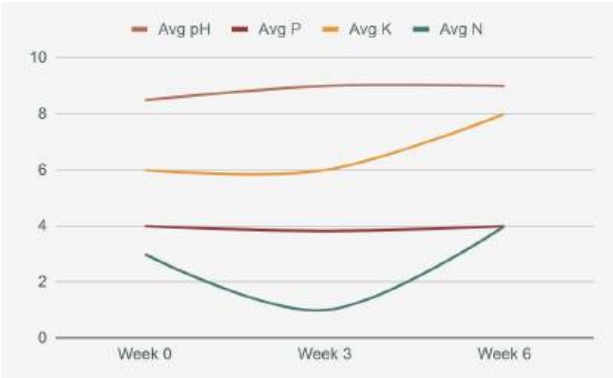


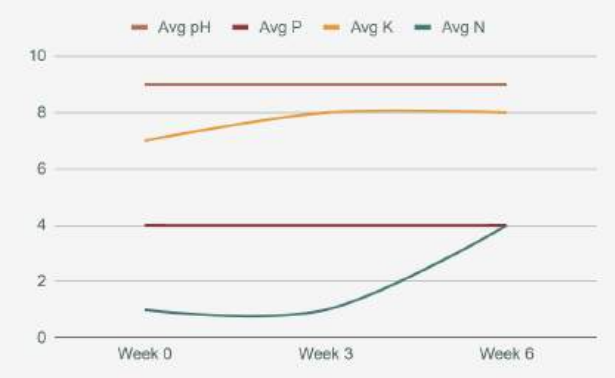


Figures 1.9 (left) & 1.10 (right) depict the potting soil group and sand-based group for asparagus, respectively. Parallel lines depict no change in the concentration of the marked nutrient.

Alfalfa

Similar to trials done with the rest of the crops, most nutrients remained roughly at their initial concentration, with little significant change. Nitrogen concentrations in Alfalfa pots resembled those of Dent Corn; by Week 3, nitrogen concentrations had dropped or remained constant for the potting soil and sand-based soil groups, respectively. By Week 6, the average nitrogen concentration had increased to a high concentration (4).





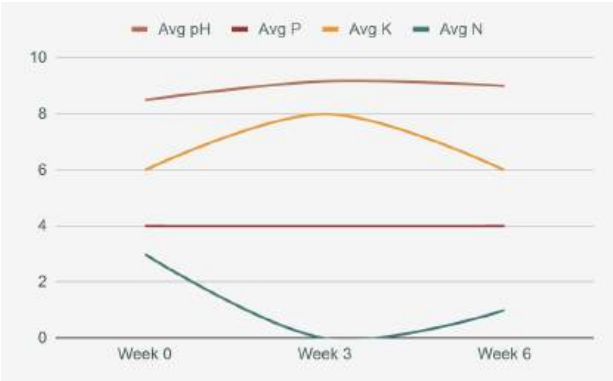
Figures 1.11 (left) & 1.12 (right) depict the graphs of nutrient data for alfalfa pots. Figure 1.11 represents data collected from pots containing potting soil, while Figure 1.12 represents data collected from pots containing sand-based soil.

In Figures 1.11 and 1.12, nitrogen concentrations both dip to trace levels at Week 3, and proceed to increase drastically to a high concentration (4), which correlates to over 60 lbs of N/acre.

Switchgrass

Switchgrass was a unique crop from the others. pH and phosphorus trends remained the same. However, the pots performed differently for potassium and nitrogen. For nitrogen, the results from the potting soil group dropped significantly, even reaching untestable levels of nitrogen during Week 3 before testing for traces of nitrogen in Week 6 (See Figures 1.13 and

1.14 below). This was different from sand-based soil, which remained at trace levels for all 6 weeks. Potassium concentrations remained very similar to their initial levels. Surprisingly, the potassium concentrations actually increased slightly, solidifying the conclusion that the soil in those pots had a very high concentration of potassium.





Figures 1.13 (left) & 1.14 (right) depict the graphs of nutrient data for switchgrass pots. Figure 1.13 represents data collected from pots containing potting soil, while Figure 1.14 represents data collected from pots containing sand-based soil.

Discussion

The data collected over the course of this study indicate that the null hypothesis is accepted for both pH and phosphorus, which showed little variation for all crop groups. However, the alternative hypothesis, which stated that there would exist different trends for different crops, is also accepted for nitrogen. Potassium concentrations did show variation from crop to crop. However, as briefly explained in the results, despite slight differences, potassium concentrations were still considered to be in the very high range. Thus, the null hypothesis is accepted with the understanding that a lengthier study must be done in order to confirm/reject the claim.

When looking at nitrogen concentrations specifically, it is clear that farmers can help replenish depleted nitrogen content best with Dent corn, alfalfa, and cowpeas in rotation with more depleting crops, such as asparagus, soybean, and red clover. Switchgrass may serve as a better nitrogen fixer in more sandy/looser soils compared to in a more nutrient-dense potting soil. This indicates more significant location-specific (different soil types) variations in nutrient spending for switchgrass. Alfalfa ended with the highest concentration of nitrogen, indicating that it could benefit the soil after a more depleting crop, such as soybean or asparagus. From the cover crops, switchgrass is the most effective in maintaining soil health, as it increases/maintains nutrients in the soil while still preventing erosion.

Additionally, for crops that quickly deplete nitrogen concentrations, results show that alfalfa increased nitrogen concentrations the most for cover crops. If more readily available, red clover can work as a suitable cover crop for farmers who primarily farm crops such as dent corn and cowpeas. This is because the red clover results showed a stable concentration of all nutrients throughout the 6 weeks. Thus, for cash crops that do not deplete the soil, it is not as necessary to

have a crop that increases the nitrogen concentration of the soil. Because of this, red clover is the most suitable cover crop for such cash crops.

Of the cash crops (dent corn, cowpeas, soybeans, and asparagus), dent corn provided the most nitrogen to the soil. Therefore, it would make most sense to put it in rotation as it is both considered a very important crop for the market, but can replenish nitrogen lost during the soybean and asparagus seasons. Cowpeas also consistently replenished/maintained nitrogen concentrations in both soil groups, helping to balance a more soybean-dense crop rotation. Ultimately, such pairings can create smaller crop rotations that maintain soil health, and thus, create stronger plants with better yields.

Implications and Limitations

For farmers, knowing how different crops affect their soils is important for decreasing maintenance on the land. This decreases the cost of fertilizers. It is also a more environmentally friendly approach to agriculture, as the data provided by this study can help Midwestern farmers adjust their farming practices to maintain long-term soil health. This research provides preliminary data for common Midwestern crops among various categories (cover crops, perennials, legumes, etc) to aid in decision-making for individual farmers. With the growing environmental and economic disparities, it is increasingly important that all farmers begin to look for adjustments they can make for better yields without increasing cost.

Despite providing some insight into how crops work with the nutrients in the soil, this experiment does not provide much information on nutrient trends beyond the seedling stage. Thus, further research must be done for a longer period of time. Additionally, because of the type of testing done to determine soil concentrations, there is more than likely a slight source of human error when performing the tests or interpreting the results. Thus, the test results are more of an estimate (range) of concentrations as indicated by La Motte's results guide. Overall, this research project aims to interpret some trends that may exist among different crops.

New Understanding

The results from this experiment for dent corn and soybeans affirm previous research from Wu, Davis, & Sohngen, which found that corn was better for soil health than soybeans. Thus, the claim that a higher corn-to-soybean crop ratio is better remains (2025). This study also provides insight into other crops and their impacts on the soil. The results indicate that nutrient concentration trends are non-linear. This differed from my initial predictions about the results. This impacts the ability to predict concentration trends for the rest of the crops' lives. The study also made it clear that certain nutrients such as nitrogen are much more variable compared to others, which explains why fertilizers are used in the field to replenish soil nutrients.

Future Research

Farmers need to begin looking at improving agricultural practices; through this study, farmers can work with the environment, reducing maintenance and cost, empowering choices

they make about their farms, and allowing for farms to benefit the environment without sacrificing their productivity. Ultimately, more experimental research is needed in order to collect detailed long-term data about several crops. Further collection of foundational data is needed to build a better understanding of how agriculture impacts the soil in the long term. Experiments that isolate variables can aid in collecting such research. Future research would be beneficial for exploring alternative farming techniques such as monocropping and *alley cropping*. Additionally, more similar research can be done to explore the effects of other variables on the soil, such as sunlight, weather, or soil additives.

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Phage-Based Biocontrol of Escherichia Coli: Characterization of a Lake Water-Isolated Bacteriophage for Application in Milk Preservation By Chloe Kim

1. Abstract

Microbial food spoilage and foodborne disease are the main challenges in the food industry. Current preservation methods are frequently associated with changes in organoleptic characteristics and loss of nutrients. For this reason, bacteriophage offers an alternative natural method as a biocontrol agent that can reduce bacterial contamination in food without altering the organoleptic properties. This study was conducted to isolate and characterize bacteriophage from lake water to control foodborne pathogenic bacteria *Escherichia coli(E. coli)*. Since E. coli is frequently found in spoiled milk, the bacteria used in this experiment were isolated directly from spoiled milk samples. Its identity as *E. coli* was confirmed through 16S rRNA gene sequencing analysis. Isolation was done by using the double agar overlay assay method. The phage efficiency was measured by comparing the plaque-forming units per milliliter(PFU/mL) for the solution with the phage and without the phage. Phage BP1 derived from the lake water showed the highest reduction in the host bacteria of *E. coli,* with a bacterial reduction of 99.99% on pasteurized milk samples at 37˚C incubation. These results demonstrate that bacteriophage BP1 can effectively inhibit *E. coli* growth in milk, suggesting its potential as an innovative and natural biopreservative in the food industry.

2. Introduction

Microbial food contamination is a major concern in the food industry. Contaminated food could contain a variety of microbes, including bacteria that can cause foodborne disease or food spoilage. Food spoilage is a major contributor to food waste and thus, to the negative environmental impacts of food production and processing. Globally, it is estimated that 15-20% of all food available at consumer levels is wasted. Among the diverse causative agents of food spoilage, *E. coli* is one of the most commonly found bacteria in many spoiled foods and can cause severe foodborne disease. It is transmitted to humans through contaminated foods, such as raw or undercooked ground meat products, spoiled milk, and contaminated raw vegetables. *E coli.* Infection can cause symptoms, including diarrhea, stomach cramping, nausea, and vomiting.

E. coli is a Gram-negative bacterium that normally inhabits the intestines of humans and animals but can become pathogenic when ingested through contaminated food or water. Pathogenic strains of *E. coli*, such as *E. coli* O157:H7, can cause severe gastrointestinal infections leading to diarrhea, abdominal pain, nausea, and vomiting, and in extreme cases, hemolytic uremic syndrome(HUS). *E. coli* contamination frequently occurs in spoiled milk and other dairy products due to improper handling, post-pasteurization contamination, or storage at inappropriate temperatures. Because of this, *E. coli* serves as a key indicator organism for milk spoilage and food safety.

Conventional food preservation methods, such as pasteurization, high-pressure processing, irradiation, and chemical or biological agents, are widely used to help improve food safety. However, those treatments are frequently associated with changes in organoleptic characteristics, loss of nutrients, and toxic side effects. Moreover, even with the variety of methods available, food spoilage is an outbreak that will continue to occur relatively often. For this reason, finding an alternative preservation method to control food spoilage is required. Therefore, developing an alternative, safe, and natural preservation method is essential to effectively control foodborne pathogens and extend shelf life.

One promising solution is bacteriophage biocontrol, which employs lytic bacteriophages to specifically infect and lyse pathogenic bacteria, thereby preventing their proliferation in food products. Bacteriophages are natural, highly specific viruses that target only bacterial cells, leaving human and animal cells unharmed. They are abundant in environments where bacteria thrive, such as soil, sewage, and freshwater sources. Unlike conventional methods, phage application in milk could be seamlessly integrated during the production process, such as milk storage tanks. Because bacteriophages are naturally occurring and non-toxic, they are safe for consumption and environmentally sustainable. Furthermore, their use can slow down spoilage after the milk is opened, extending shelf life and reducing economic and environmental losses.

Based on these considerations, this study aimed to isolate and characterize bacteriophages from lake water samples for use as a biocontrol agent against *E. coli* in milk. By investigating the lytic activity and preservation potential of the isolated bacteriophage, this research explores its applicability as a novel and natural preservative in the food industry.

3. Methods

Bacteriophages were isolated from lake water, then enriched and purified. Characterization of the isolated bacteriophages, including titer and the turbidity assay was determined.

Sample Collection

Samples were collected from lake water near an organic waste disposal site for bacteriophage isolation. These environments were selected because they are rich in microbial activity and likely to contain phages targeting *E. coli.*

To obtain bacterial isolates, spoiled milk samples were also prepared. Pasteurized milk was stored at room temperature(approximately 25˚C) for more than two weeks until spoilage occurred, indicated by curdling and odor changes. Then, 500μl of the spoiled milk was spread onto Luria Bertani(LB) agar plates and incubated overnight at 37˚C. After incubation, a single bacterial colony was picked and transferred into 10mL of LB broth. The culture was grown overnight in a shaking incubator at 37˚C and 120rpm. The resulting *E. coli* strain was confirmed by 16S rRNA gene analysis and was labeled N1 for its use as the host bacterium.

Bacteriophage isolation

E.coli N1 was grown in LB broth to mid-log phase by incubation at 37 °C, 120 rpm. 1ml of lakewater sample and 300 µL of each bacteria culture were added into 30 mL of LB broth. The samples were supplemented with 100 µL of 10 mM of CaCl2 and 100 µL of 0.5 mM of MgSO4 to enhance the bacteriophage growth and were incubated at 37 °C, 150 rpm, overnight. The samples were then centrifuged at 7000×g for 15 min. The supernatant was filtered using a 0.22 µm pore-size disposable syringe filter to remove the remaining bacterial cells. The filtrate was centrifuged again at 7000×g for 10 min and tested for the presence of bacteriophages using the agar overlay assay. Agar overlay assay was done by pouring top agar (LB consist of 0.6% agar) to the bottom agar (LB consist of 2% agar), where 150 µL of bacteriophage filtrate, 150 µL of mid-log phase bacterial host culture, 50 µL of 10 mM CaCl2, and 50 µL of 0.5 mM MgSO4 were mixed in 4 mL of molten LB soft agar and poured onto LB agar plate, then followed by incubation at 37 °C overnight. Clear plaque formation was observed.

Primers design and colony PCR

Primers targeting hypervariable regions of the E. coli 16S rRNA gene were developed by using SnapGene. Two sets of primer pairs were designed and tested: E. coli Test Top(forward, targeting bases 284 to 302; 5'-CCTAGCTGGTCTGAGAGGA- 3') and E. coli Test Bottom(reverse, targeting bases 1401 to 1416; 5'-GGGCAGTGTGGTACCC-3'). The optimal melting temperature and expected PCR product sizes for the primer pairs were 56.5˚C and 1133bp.

A single colony was picked with a pipette tip by dropping a small amount of sterile water. The picked colony was put on an LB plate. The bacteria were lysed to release the DNA by directly adding a small volume of the sample to the PCR reaction. The PCR colony was then verified by testing the PCR colony's primers. The PCR colonies were run on an agarose gel to determine their size.

Bacteriophage purification and enrichment

The isolated lytic bacteriophages were purified by stabbing the clear plaque gently using a sterile tip. The tip was then placed into 10 mL of LB Broth and pipetted up and down to release the bacteriophage particles. Bacteriophages were enriched by adding 250 µL of mid-log phase bacterial host culture into the LB Broth and incubating at 37 °C overnight at 120 rpm. After enrichment, the mixture was centrifuged at 7000×g for 15 min and the supernatant was filtered using a 0.22 µm pore-size membrane filter to obtain bacteriophage stock.

Bacteriophage Titer Determination

Titers were determined using the agar overlay assay method. A series of tenfold dilutions of bacteriophage lysate solution were made using SM buffer (50 mM Tris-hydrochloride (Tris–HCl) [pH 7.5], 0.1 M NaCl, 8 mM magnesium sulphate heptahydrate (MgSO4•7H2O) and 0.01% (w/v) gelatine). Each dilution was plated according to the agar overlay assay and incubated at 37 °C overnight. The number of visible plaques were calculated between 50 and 300

plaques which was expressed as plaque forming unit per milliliter (PFU/mL). The titer was calculated using the following equation:

$$Titer(PFU/mL) = \frac{Number\ of\ Plaques}{Volume\ Plated(mL) \times Dilution\ Factor}$$

Bacteria growth reduction assay

Overnight grown host bacterial cultures($1 \times 10^9 CFU/mL$) were added to two LB broth flasks. One flask was inoculated with phage($1 \times 10^{10} PFU/mL$) while the other contained no phages as a control. Both flasks were incubated at 37˚C, shaken at 120rpm. The Optical Density(OD_{600}) was calculated at 30-minute intervals for 2 hours in total. The presence of resistant colonies was confirmed by increasing the OD_{600}.

Colony counting assay

To evaluate the bacteriophage's preservation potential in milk, *E. coli* N1 was mixed with phage BP1 in milk and stored under refrigeration(4˚C) for six days. As a control, *E. coli* N1 was added to milk without phage. Every two days, a small amount of each milk sample was collected, serially diluted with sterile distilled water and spread onto LB agar plates. Plates were incubated overnight at 37˚C and the resulting colonies were counted.

The number of viable bacterial cells was expressed as colony-forming units per milliliter(CFU/mL) using the following equation:

$$Colony\ Count(CFU/mL) = \frac{Number\ of\ Colony}{Volume\ Plated(mL) \times Dilution\ Factor}$$

Positive control samples contained only *E. coli* N1, while negative controls contained only bacteriophage lysate.

4. Results

Isolation and Detection of Host Bacteria and Lytic Bacteriophages

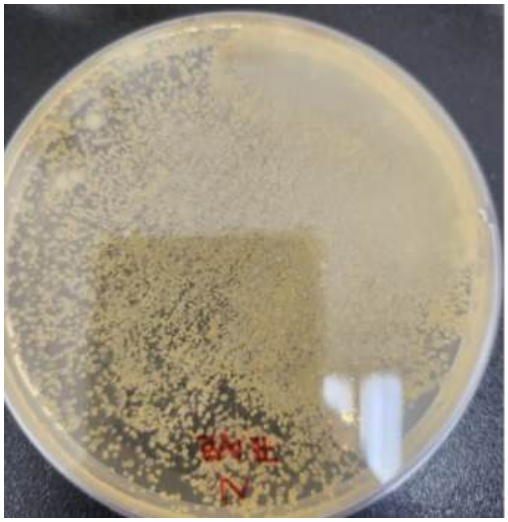


Fig 1: E. coli isolated from spoiled milk

We isolated E. coli from spoiled milk. After overnight incubation at 37˚C for a day, small, yellow spots called colonies of the host bacteria appeared on the LB agar plate(Figure 1). To confirm that the bacteria isolated from the spoiled milk are E. coli, we compared the DNA sequences of E. coli 16S rRNA gene and the sample extracted from the spoiled milk in Figure 1.

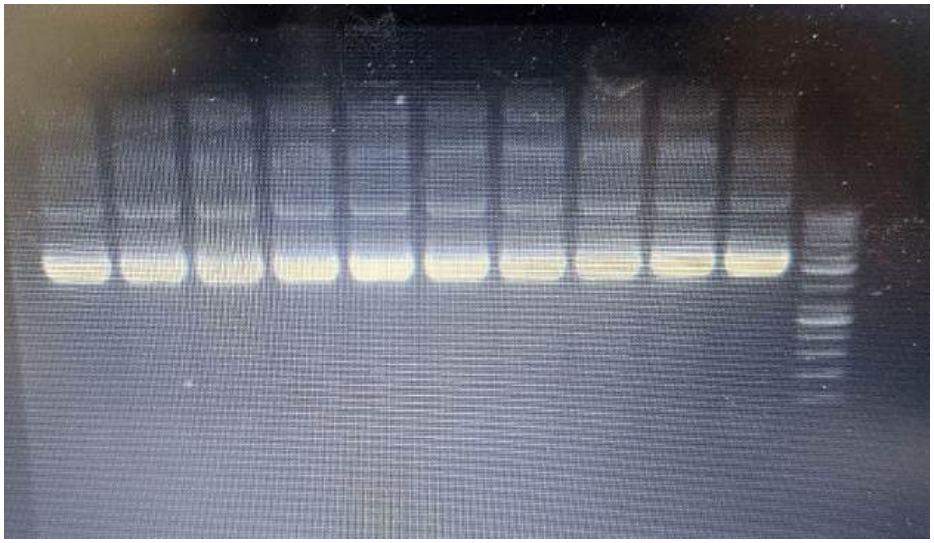


Fig 2: Agarose Gel Separation of the PCR mixtures

After conducting PCR on the sample from the isolated bacteria , the agarose gel separation of the PCR mixture created a 1200bp DNA ladder.

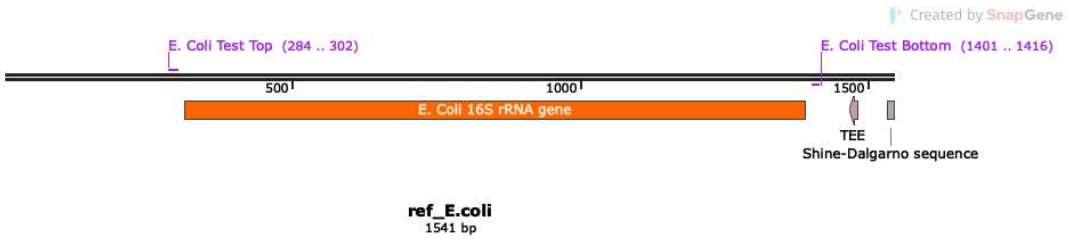


Fig 3: Primer designed in SnapGene

The top and bottom primers showed to have a similar melting temperature and the expected PCR size as noted above in the methods section.

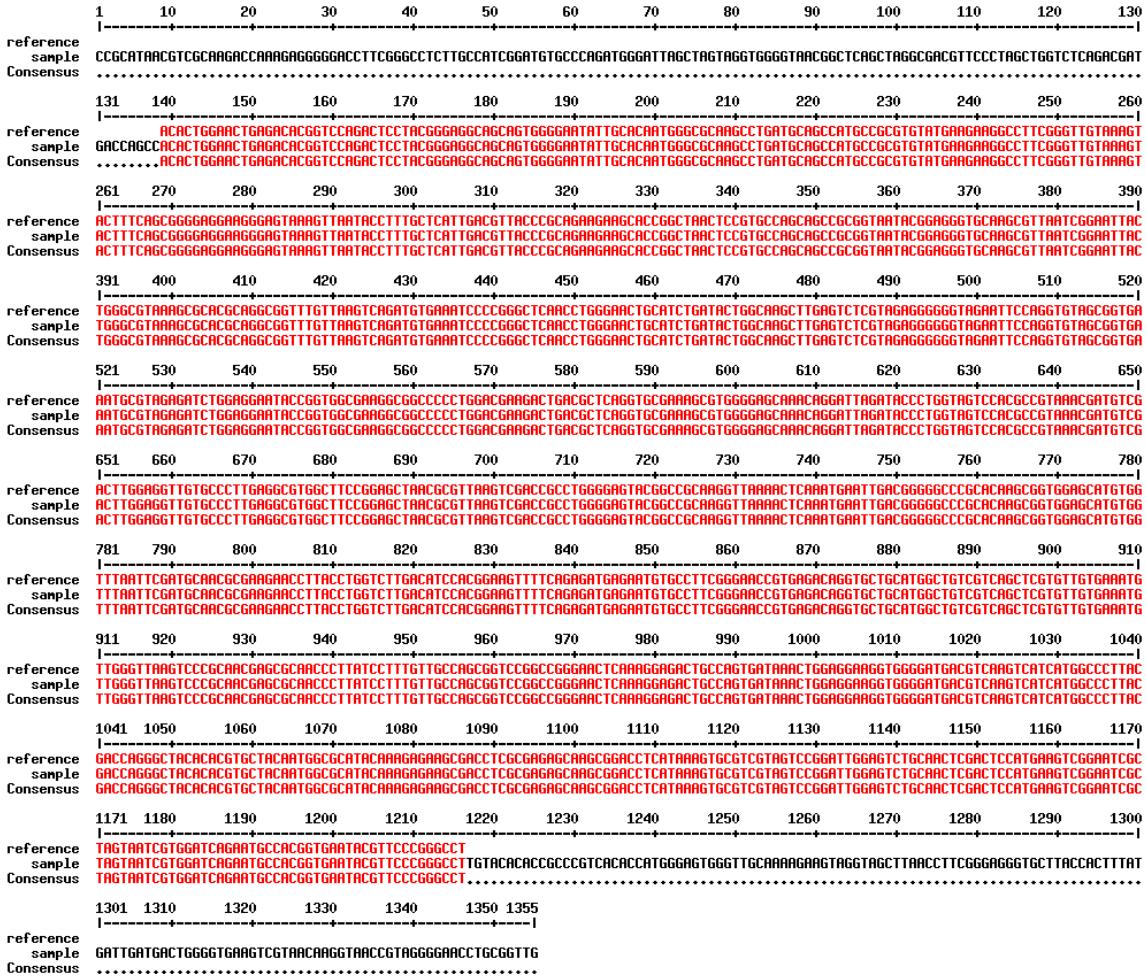


Fig 4: DNA Sequence Alignment Result

We also examined the specific DNA sequence alignment between the reference and the sample solutions by using MultAlin to look for any mismatches. No mismatches appeared from 284 to 1416bp and thus, resulted in 100% alignment of the two sequences, confirming that the sample extracted from the spoiled milk contains E. coli 16S rRNA gene.

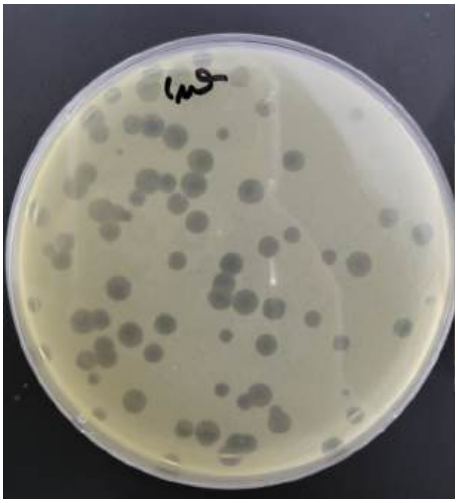


Fig 5: Bacteriophage isolated from the lake water

After overnight incubation with N1 E. coli, the lake water sample showed multiple spots of the host bacteria, which suggests that it contained a higher number of active phages than the lakewater sample, showing no spots of bacteria(Figure 5). There were plaques of various sizes, and we selected the largest one. Since strong lytic activity indicates a higher ability to kill bacteria, we chose the single largest plaque. . The plaques were 4-5mm in diameter, which is typical of lytic phages. After purifying the phages through several rounds of testing and culturing the bacteria, we measured their concentration and found a final titer of 1.2×10^{11} PFU/mL (plaque-forming units per milliliter), indicating a very strong phage preparation.

Dilution Step	Dilution Factor	Volume of Phage Solution(µl)	Volume of Diluent Solution(µl)	Using Volume(µl)	Volume Factor
1	10^{-1}	100	900	100	10
2	10^{-2}	100	900	100	10
3	10^{-3}	100	900	100	10
4	10^{-4}	100	900	100	10
5	10^{-5}	100	900	100	10
...	...	...	...	...	...
10	10^{-10}	100	900	100	10

Fig 6: Dilution and Volume Factors Table

To perform the phage reduction assay, the infectivity between the isolated bacteriophage BP1 and its bacterial host N1 was first verified. The bacterial concentration (density) of N1 was

measured by optical density (OD) readings to estimate the number of bacterial cells, while the phage concentration (titer) of BP1 was determined. Based on these measurements, the multiplicity of infection (MOI) was calculated as 10 for subsequent assays.

This figure confirms that both the bacterial and phage concentrations were accurately determined prior to testing phage infectivity, ensuring reliable conditions for the reduction assay.

Time (minutes)	Negative Control	Negative Control	Phage Treated
0 min	0.714	0.714	0.714
30 min	1.032	1.16	1.032
60 min	0.98	1.348	0.98
90 min	0.1	1.772	0.1
120 min	0	1.804	0

Fig 7: Change in E. coli density (OD_{600}) over time with and without phage treatment

Figure 7 presents the measured bacterial turbidity at 30-minute intervals during the reduction assay. The purpose of this assay was to confirm whether BP1 could reduce the N1 bacterial population. Bacterial reduction was quantified by colony-forming unit (CFU) counts, allowing the evaluation of phage infectivity over time. The data table displayed in the figure illustrates the gradual changes in bacterial density at each time point.

As shown in Figure 7, bacterial density in the control group (without phage) increased continuously, indicating normal bacterial growth. In contrast, the phage-treated culture showed an initial slight increase up to 30 minutes, followed by a rapid decline after 60 minutes, and complete clearance by 120 minutes. This demonstrates that BP1 phage effectively lysed E. coli cells, confirming strong infectivity and reduction capability.

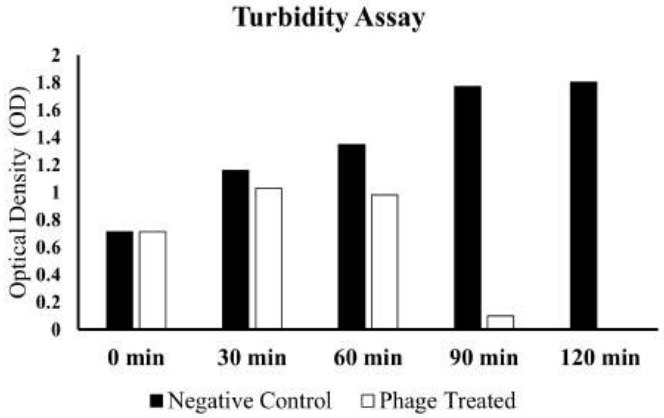


Fig 8: Effect of phage treatment on bacterial turbidity over time

Figure 8 visualizes the results summarized in Figure 7. Compared to the control group (without phage), bacterial density in the phage-treated sample increased at a slower rate up to 60 minutes. After 60 minutes, a sharp decline in bacterial density was observed, indicating that BP1 effectively infected and lysed the host bacteria after sufficient incubation time. These results confirm BP1's infectivity and its ability to significantly reduce bacterial populations under controlled conditions. These results confirm that BP1 phage maintained strong lytic activity in milk and was capable of reducing bacterial contamination even under refrigerated storage conditions.

Phage Efficacy in Milk at Different Times

To test whether BP1 phage could function as a natural preservative in a realistic food matrix, the phage was added to pasteurized milk samples artificially contaminated with E. coli at MOI 10. The samples were stored at 4 °C to simulate refrigerated conditions.

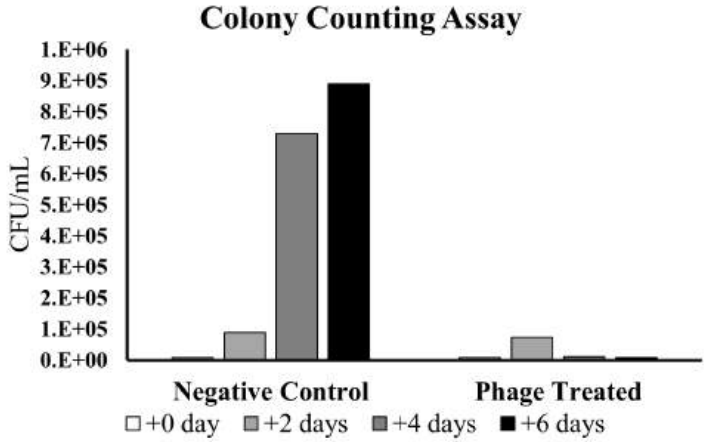


Fig 9: CFU counts of negative control and solution with phage at 4˚C

Building on the previous experiments, Figure 9 investigates whether BP1 could also reduce bacterial contamination in milk samples under refrigeration (4 °C), simulating real storage conditions. The phage was applied to contaminated milk samples at an MOI of 10, identical to the bacterial reduction assay conditions.

In refrigerated milk samples, bacterial populations showed similar growth patterns between control and phage-treated groups during the first two days. By Day 4, however, the BP1-treated samples exhibited a notable reduction in bacterial count, mirroring the reduction pattern observed in the in vitro assays. This demonstrates that BP1 remains active and capable of bacterial reduction even under low-temperature conditions, indicating potential for use as a natural food preservative in refrigerated environments.

5. Discussion

The results of this study demonstrate that lytic bacteriophages isolated from environmental sources such as lake water can be highly effective in reducing Escherichia coli

populations in pasteurized milk. The application of bacteriophages at an optimized multiplicity of infection (MOI) of 10 led to a significant bacterial reduction exceeding 99.999% within 2 hours in laboratory broth conditions and up to 99.97% in real food matrices at ambient storage temperatures. These findings support the feasibility of using phages as natural, targeted biocontrol agents for improving food safety without altering product quality.

The observation that lake water-derived phages exhibited high lytic activity against E. coli is consistent with previous studies that identify lake water as a rich reservoir of bacteriophages due to the dense microbial diversity it supports (Rohde et al., 2018). The successful isolation and amplification of phages from compost-rich lake water reinforces its utility as a sampling site for future phage discovery initiatives.

Efficacy of Phage Biocontrol in Milk

Phage application in milk showed substantial efficacy, where bacterial load was reduced by over 99% within 24 hours. This finding correlates with previous research, such as Garcia et al. (2022), which observed similar phage-mediated suppression of E. coli in dairy products. The increased effectiveness at room temperature is likely attributed to the enhanced metabolic activity of E. coli, which supports more efficient phage replication and subsequent host lysis (Hagens & Loessner, 2010).

Comparison with Conventional Food Preservation Methods

Compared to traditional preservation techniques such as pasteurization, chemical additives, and irradiation, bacteriophage-based preservation offers several distinct advantages. Most notably, phages demonstrate host specificity, meaning they selectively target E. coli without disrupting the sensory or nutritional properties of the food or affecting beneficial bacteria (Goodridge & Abedon, 2003). Pasteurization, although effective, may degrade heat-sensitive vitamins and alter milk flavor (Macdonald et al., 2011). Chemical preservatives, on the other hand, face increasing consumer resistance due to concerns over toxicity and allergenicity (Greer, 2005). Phage therapy, being natural and residue-free, presents a promising alternative that addresses both food safety and consumer health concerns.

The isolated phage itself is active specifically against the dominant E. coli strain which makes it advantageous in minimizing collateral damage to non-target microorganisms. But this can also limit broader-spectrum applications unless phage cocktails or genetically engineered polyvalent phages are employed (Chan et al., 2013).

Limitations and Challenges

While the results were promising, several limitations must be acknowledged. The study focused only on pasteurized milk, and the performance of phages may vary in other foods depending on their pH, viscosity, fat content, or nutrient availability. In addition, phage activity can be affected by temperature, and in this project, all experiments were conducted only at room temperature and refrigerator temperature. Further studies would be needed to evaluate whether

phages remain effective under refrigeration or other storage conditions commonly used in food production and retail environments. Another limitation was the use of only one E. coli strain for testing. In real-world settings, contamination may involve multiple strains or even different bacteria altogether. Addressing this issue would require broader testing and the development of phage mixtures capable of targeting a wider range of pathogens.

Future Changes

In future studies, it would be valuable to examine how phages perform in different types of food and under various environmental conditions. Additional tests could also include genomic sequencing of the phages to confirm their safety and stability. Moreover, combining phages with other natural antimicrobials such as bacteriocins or organic acids may lead to stronger and more reliable food preservation systems.

Overall, this study supports the idea that lytic bacteriophages are a powerful and natural tool for reducing harmful bacteria in food. Their precision, effectiveness, and environmental safety make them a compelling alternative to chemical preservatives. As more research continues, phage-based methods may become a regular part of food safety practices, especially for products where consumers prefer clean, additive-free labels.

6. Conclusion

This study successfully isolated and characterized a lytic bacteriophage, BP1, capable of effectively targeting and reducing Escherichia coli contamination in both laboratory media and milk samples. The bacteriophage demonstrated a 99.99% reduction in bacterial populations under optimized conditions and maintained activity even at refrigeration temperature (4 °C), confirming its potential as a natural biocontrol agent. Compared to conventional food preservation methods, phage-based preservation offers a highly specific, eco-friendly, and non-destructive approach that preserves the nutritional and sensory qualities of food. Although further research is required to evaluate its efficacy across various food types, storage conditions, and bacterial strains, these findings provide strong evidence that bacteriophage BP1 could serve as a promising candidate for sustainable food preservation and safety enhancement in the dairy industry and beyond.

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Dysregulated Immune Activation: A Proposed Mechanism for Linking Myasthenia Gravis And COVID-19 Vaccinations By Meena Basnayake (student)[1], Huan-Ting Lin (mentor)[2*]

Abstract

Viral vaccines play a critical role in disease protection against viral infections by training the immune system through the induction of humoral and adaptive responses. Paradoxically, vaccines could induce non-specific activation of the immune system response, triggering potentially fatal relapses in autoimmune disease. Myasthenia Gravis (MG) is a stereotypical autoimmune disease susceptible to initiation or exacerbation following vaccine administration. This paper conducts a review of published data, suggesting a link between COVID-19 vaccine administration and subsequent initiation or exacerbation of MG-related symptoms. Based on current knowledge of the immune system, we propose autoantibody production driven by non-specific activation of memory B cells as a mechanism for triggering MG initiation or causing relapse. Knowing underlying risk factors would facilitate the establishment of effective disease management strategies.

Keywords

Myasthenia Gravis, SARS-CoV-2, Autoimmune Disease, Immune Response.

Introduction

Autoimmune Disease and Myasthenia Gravis

Autoimmune diseases can be described as a case of mistaken identity where one's immune system inadvertently attacks healthy tissue or organs. These are often triggered by complex environmental and biological factors, leading to chronic inflammation, tissue damage, and damage to affected organs. In many cases, there is no curative treatment available, hence patients have a lifelong predisposition to the effects of these diseases.[1] The underlying disease-initiating mechanisms of MG remain unclear, but there is evidence suggesting that disease onset or relapse strongly correlates with infections, medications, and dysregulated immune responses (flare-ups, periods of worsening symptoms).[2] Myasthenia Gravis (MG) is a prototypical autoimmune neuromuscular disorder because biological mechanisms pertaining to dysregulated autoimmunity have been clearly defined. It is characterized by skeletal muscle weakness and fatigue, caused by impaired synaptic transmission of acetylcholine at the neuromuscular junction (NMJ), where nerve cells communicate with muscle fibers.[3] This disruption impairs the transmission of nerve signals, leading to muscle weakness that impacts eye movements, facial expression, swallowing, and, in severe cases, breathing.[4] During severe

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flare-ups, patients can experience a “myasthenia crisis,” which is a potentially life-threatening case of respiratory failure and requires emergency care. Myasthenia crises are most commonly triggered by infections such as pneumonia or viral illnesses. Still, they can also be triggered by physical and emotional stress, certain medications (antibiotics, beta blockers, anesthetics), surgery, pregnancy, or hormonal changes. Early identification and treatment are critical to preventing irreversible complications.

Discussion

Myasthenia Gravis Disease Background

Two major subtypes of MG have been recognized and identified based on the presence of specific autoantibodies targeting either acetylcholine receptor (AChR-MG) or muscle-specific kinase (MuSK-MG)[5] (Figure 1). In both instances, autoantibodies increase significantly in the bloodstream produced by antigen-specific plasma B cells. The underlying immunological mechanisms differ, but the symptoms generally overlap. At the neuromuscular junction, muscle fibers normally express far more AChRs than are needed for normal, effective signal transmission. This excess is known as the neuromuscular safety margin, and this allows the synapse to continue to function even if some receptors become damaged or temporarily unavailable.[6] In MG, however, antibody binding, complement-mediated injuries, or receptor internalization can significantly reduce the number of functional AChRs. Under normal conditions, this safety margin allows the neuromuscular junction to tolerate small fluctuations in antibody levels without any harm. However, when antibody-mediated damage exceeds this threshold, neuromuscular transmission fails, which then results in muscle weakness. Even small amounts of autoantibodies can trigger muscle weakness, fatigue, and, in severe cases, myasthenia crises.[7]

The more common of the two, AChR-MG, which affects over 90% of MG patients, occurs when IgG1 and IgG3 antibodies produced by B cells bind to acetylcholine receptors (AChRs) on the membrane of muscle cells. These antibodies interfere with normal muscle function in several ways. First, they can activate the complement system, which causes lysis of the postsynaptic muscle membrane, thus reducing the number of AChRs and impairing neuromuscular signal transduction.[5] In severe cases, this can contribute to systemic organ stress and damage through increased release of cytokines and injuries in tissues. Secondly, the antibodies can bind to and clump multiple AChRs together, leading to their internalization from the surface of the muscle cell in a process known as receptor internalization (removal and degradation of the receptors). This further reduces the number of available receptors on the muscle membrane, diminishing its ability to receive nerve signals and weakening signal transmission.

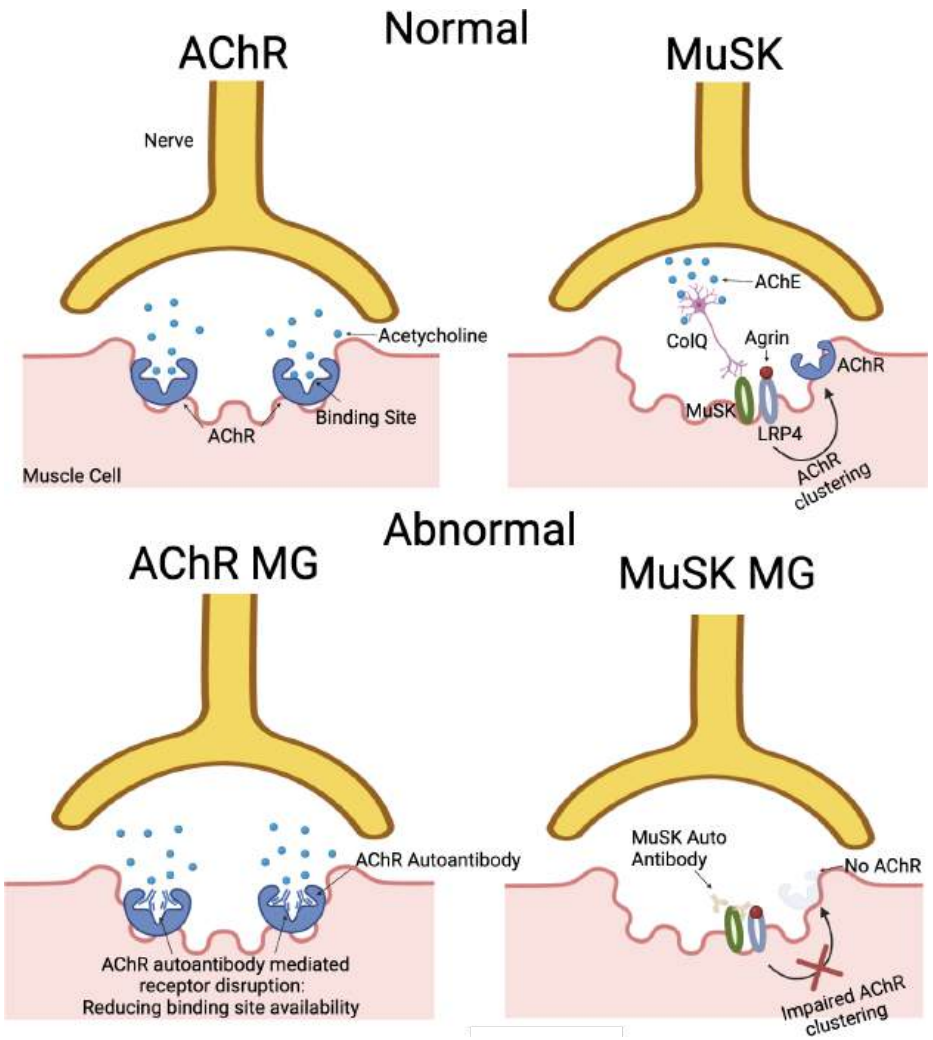


Figure 1. Mechanism of acetylcholine receptor and MuSK antibodies mediated Myasthenia Gravis (AChR-MG).) In healthy neuromuscular transmission, acetylcholine released from the nerve binds to acetylcholine receptors (AChRs) on the muscle cell, enabling normal muscle activation (top left panel). In AChR-MG, IgG1 and IgG3 autoantibodies bind to AChRs, blocking acetylcholine-mediated activation of AChR, damaging the cell membrane, and thus, leaving fewer AChRs on the muscle surface. This results in muscle weakness (bottom left panel). MuSK MG pathology – (top right panel) Agrin released from motor neurons binds to LRP4 and activates through Dok7 signaling, leading to AChRs clustering. This clustering is essential for stable synapse formation, which leads to efficient neuromuscular transmission. (right panel) In MuSK-MG, IgG4 autoantibodies disrupt this pathway and the LRP4-MuSK interaction, impairing AChR clustering and thus weakening neuromuscular transmission.[8] Overall, both AChR-MG and MuSK-MG impair neuromuscular transmission through distinct antibody-mediated mechanisms. Despite targeting different molecular pathways, both forms of MG reduce the efficiency of acetylcholine signaling at the neuromuscular junction, ultimately resulting in muscle weakness.

In contrast, MuSK-MG accounts for roughly 5-8% of MG cases[9] and targets the muscle-specific kinase. MuSK is a protein essential for aligning AChRs at the NMJ. This MG subtype is driven primarily by IgG4 antibodies that are produced when defective B cells escape immune checkpoints, which are the mechanisms that normally eliminate autoreactive cells. T cells also play a role in MuSK-MG, particularly Th1 and Th17 subsets,[10] which promote B cell activation and contribute to increased IgG4 production. Additionally, these T cells enhance inflammatory responses, further intensifying the autoimmune response towards MuSK. It is important to note that killer and memory T cells can also become activated and expand in a non-antigen-specific way. For example, during viral illness, they can indirectly exacerbate autoantibody production by providing excess cytokine support to autoreactive B cells, even without antigen-specific targeting.[11] Therefore, this non-specific activation could further exacerbate MuSK-MG in susceptible patients.

In both types of MG, autoantibody titer levels in the bloodstream are noticeably raised but do not always correlate clearly with disease severity. For AChR antibodies, a normal value is ≤ 0.02 nmol/L, while results less than 0.4nmol/L are considered positive. In many generalized MG patients, titers range from 1 to 100nmol/L, though some severe causes can pass 200nmol/L.[12] Studies show that reductions in AChR-Ab titers over time often show clinical improvement, and therefore can serve as a useful biomarker of treatment response. In addition, memory B cells are also important predictors of disease severity, persistence, and relapse. These cells are often identified by markers such as CD19 and CD27. MG patients frequently show expanded populations of CD19+/CD27+ memory B cells compared to healthy individuals,[13] which produce autoantibodies against AChR or MuSK.

For most patients, MG can be managed through a combination of lifestyle management strategies and medications such as immunosuppressants. Patients can lead perfectly normal lives but remain highly susceptible to developing life-threatening complications. Treatment for MG focuses mainly on improving muscle strength and suppressing abnormal immune responses. First-line therapies include acetylcholinesterase inhibitors such as pyridostigmine, which enhance the communication between nerves and muscles. These treatments are symptomatic and help with the temporary replacement of muscles, but do not cure the disease itself.[14] Other treatment types include immunosuppressants such as corticosteroids and cyclosporine, which are used to reduce the overall autoantibody production by the overactive B and T cells.

Although precise disease-initiating mechanisms are not well understood, it is known that in MG, there are long-term antigen-specific memory B and T cells that persist in the bloodstream of patients. Disease relapse occurs following B-cell-mediated autoantibody production against AChR and MuSK. The classic pathway of activation for these memory cells is through the adaptive immune response. However, non-specific activation of these memory cells could occur, and when anti-AChR antibody-producing B cells are implicated, relapse or exacerbation of MG occurs.[15] Recent studies have explored the relationship between COVID-19 vaccines and MG severity. While vaccinations do not directly cause MG, researchers have documented rare cases of symptoms worsening shortly after patients received their vaccines, possibly due to temporary

immune activation that enhances these already existing autoreactive B and T cell activity.[16] Therefore, factors that could trigger aberrant activation of the immune system have the potential to trigger new onset MG for the first time or cause relapse.

Clinical Reports of Autoimmune Flares Relating to COVID Vaccinations

The rapid global spread of COVID-19 led to the widespread use of newly developed vaccines designed to target SARS-CoV-2. There has been increasing data to show that administration of COVID-19 vaccines has the potential to trigger autoimmune reactions in susceptible patients. One of the largest efforts to evaluate this came from the COVAD study, an international survey of more than 15,000 participants across 90 countries. It found that although most patients tolerated vaccination well, 16.3% of patients reported post-vaccine flares, compared with only 4.2% of healthy controls. Interestingly, occurrence was most common in patients with autoimmune diseases like MG, such as systemic lupus erythematosus, rheumatoid arthritis, and psoriasis. Most of these flares could be resolved without major intervention, but a proportion required further treatment. Overall, it indicates that vaccinations have the potential to trigger adverse immune reactions in vulnerable groups.[17] In another study, involving more than 2,300 patients with autoimmune rheumatic diseases (including rheumatoid arthritis, psoriatic arthritis, and spondyloarthritis), 21-24% of patients experienced disease flares following mRNA COVID-19 vaccination, and 10-15% of these flares were classified as severe. Researchers highlighted that patients with higher baseline disease activity and activated immune cells were most vulnerable.[18] It raises some key questions, most notably, what is the relationship between vaccine administration and disease initiation, and what could be the underlying disease mechanisms?

Worldwide Distribution of Vaccination-Triggered MG

A systematic review of 11 studies (#1 Table 1) and a pooled analysis of 258 neuromuscular disease cases (22 Myasthenia Gravis) found that post-vaccine MG events occurred after administration of both mRNA vaccines (Pfizer-BioNTech and Moderna) and vector-based vaccines (Oxford-AstraZeneca and Johnson & Johnson)[19] (#2 Table 1). Symptoms generally appeared within one to two weeks, involving ocular and bulbar muscles. There was even progression to myasthenic crisis, which could be life-threatening if it is not managed appropriately. Most patients had repetitive nerve stimulations, and most were AChR-antibody positive, but improved with standard treatments such as steroids, IVIG, or pyridostigmine, although severe outcomes such as myasthenic crises were observed in 3 cases.

Furthermore, there have also been several individual case reports involving other vaccine types as well. In one case, a 68-year-old with no prior history of MG developed new-onset MG following an inactivated vaccine (Sinopharm) with bulbar weakness and elevated AChR titers[20] (#3 Table 1). Another case described a 37-year-old woman who developed generalized MG three days after her second Sinopharm dose. Blood levels of AChR-AB were more than eight times the

normal range[21](#4 Table 1). These reports are of particular significance because neither patient displayed MG symptoms prior to vaccination.

It is worth noting that inactivated vaccines contain inactivated viral particles and adjuvants, which are well-known non-specific inducers of B and T cells, offering insights into potential mechanisms triggering adverse MG events. It may increase the likelihood of activating autoreactive memory B cells to AChR or MuSK, providing a plausible explanation for why rare MG cases have appeared after inactivated vaccines like Sinopharm. Notably, Sinopharm-associated MG cases have been consistently rapid (typically occurring within ~3 days of vaccination), whereas cases linked to mRNA or vector vaccines display a much wider onset range (from 2 days up to 2-3 weeks).[21] In another case, diplopia and limb weakness developed after vaccination and worsened within a week, eventually requiring immunosuppressive therapy. Furthermore, a case series involving seven patients with new-onset myasthenia gravis (six Pfizer, one Moderna) further raised concerns. Disease onset was rapid, and antibody levels were significantly raised in most cases[22] (#5 Table 1). Overall, there is a consistent pattern of new onset of MG or flare-ups that could be triggered by vaccine administration.

Table 1. Summary of five published case studies, as well as worldwide studies and datasets examining the relationship between COVID-19 vaccines and MG. This table includes systematic reviews, pooled analyses, individual case reports, and a small case series. Details shown are vaccine types, patient demographics, onset timing, antibody status, clinical symptoms, and treatment outcomes. Collectively, these reports show a temporal association between COVID-19 vaccination and rare cases of MG onset or flare-ups.

	Study/ Report	**Vaccine Type**	**Patient(s)**	**Onset Timing**	**Antibody Status**	**Symptoms**	**Treatment & Outcome**
(#1)	Systematic review of 11studies: 7 patients across 7 countries (i.e, US, Japan, Italy)	mRNA, Viral Vector	Mostly men, over the age of 50	1-21 days (avg ~7)	mixed	Ocular/bulbar weakness; 3 crises	Steroids, IVIG pyridostigmine~ > recovery
(#2)	Pooled Analysis (258 cases; 17 MG)	31% Received mRNA, 69% received a vector-based vaccine	17 MG patients (out of all patients, median was 58 years, range was from 14~90)	≤7 days in most	Mostly AChR+	New Onset & flares	Standard therapies ~> effective
(#3)	Case report #1 (individual)	Inactivated Virus Vaccine	68-year-old male	3 days post 2nd dose	AChR+ (30nmol/L)	Ptosis, dysphagia, nasal speech	Corticosteroids, IVIG, pyridostigmine ~> improved
(#4)	Case report #2 (individual - Iran)	Inactivated Virus Vaccine	37-year-old female	3 days post 2nd dose	AChR- AB titer = 3.3 nmol/L	Diplopia, limb weakness	Required immunosuppres sive therapy
(#5)	Case series (7 patients 35-80 years)	mRNA	Median age 65	3-14 days	6/7 AChR+	EMG-confirmed MG	Standard therapies: all recovered

(#6)	Keio University MG Clinic (2021) 343MG patients (294 vaccinated)	mRNA	3 female, early onset MG	2-3 days after 1st dose (2 cases), 14 days after 2nd dose (1 case)	Antibody titers (AChR, MuSK) remained stable ~> suggests immune activation, not antibody surge	MG flare-ups occurred but were rare; all improved with standard treatment	NA
(#7)	Japan MG Registry (JAMG-R) 1710 MG patients	Multiple COVID-19 vaccines (not specified per patient)	Broad national cohort	Not specified	NA	Rare vaccine-related MG flare-ups observed	NA
(#8)	Europe (Italy, Spain, UK, multicenter studies)	Primarily mRNA; some viral vector	Several hundred MG patients across three national cohorts	Within 1-2 weeks after vaccination	Antibody titers (AChR, MuSK) are typically stable, and no fatalities have been reported	MG flares were uncommon and generally mild	NA
(#9)	Middle East MG Case Series (Turkey, Iran, Qatar)	Mix of mRNA, Viral vector, inactivated	Multiple small case series (approx. 10-20 total cases across reports)	Mostly within a few days to 2 weeks after vaccination	NA	Flares occurred primarily in patients with unstable disease; Iranian centers reported several new-onset MG cases.	All cases improved with standard therapy (IVIG, steroids, pyridostigmine)
(#10)	China Neuromuscular Clinic Reports National registry level observations (<1% of MG patients affected)	Inactivated Vaccines	Mix of adult MG patients	A few days to within 2 weeks post vaccination	NA	Unlike mRNA In the dominant regions, China saw more initial MG presentation than flares	Occasional new onset MG cases, typically reversible with standard immunotherapy
(#11)	Population-based pharmacovigilance study (Bahia, Brazil) (10,169378 adults (first dose recipients)	Inactivate d, viral vector, mRNA	The majority were female, and their ages ranged from about 30 to 49	Reported after first dose (specific timing not included)	NA	AEFI prevalence : 74.3 per 100,000 doses. Predomin antly non-serio us adverse events	

In Japan, researchers from the Keio University MG clinic followed 343 patients during the 2021 vaccination period. Three female patients with early-onset MG experienced flares, all resolving with treatment, but without rises in AChR or MuSK antibodies (#6 Table 1).[22] Similarly, the Japan MG Registry (JAMG-R), which surveyed 1710 patients, reported that vaccine-associated flare-ups were not common but very much possible (#7 Table 1). Together, these findings suggest that while COVID-19 vaccines were broadly safe for MG patients in

Japan, immune activation from vaccination may have been a trigger in some cases.[23] In Europe, data from multicenter studies in Italy, Spain, and the UK consistently reported vaccine-associated MG exacerbation. Most flares occurred within the first two weeks after mRNA vaccination and were usually mild, responding well to pyridostigmine or short corticosteroid courses (#8 Table 1).[16] In the Middle East, case studies from Turkey, Iran, and Qatar found that flares occurred mainly in patients with unstable MG, recent infections, or those receiving immunosuppressive therapy such as prednisone, azathioprine, or rituximab. Iranian centers also described several cases of new onset MG following Sinopharm vaccination, with patients showing elevated AChR antibody titers but recovering fine with standard therapy (#9 Table 1).[24] In China, where inactivated vaccines such as Sinopharm and CoronaVac were predominantly used, national neuromuscular clinics reported occasional new-onset MG cases shortly after vaccination. However, unlike mRNA-heavy regions, post-vaccine cases in China disproportionately involved new-onset MG rather than flares of pre-existing disease, suggesting that different vaccines can affect the type of MG event observed (#10 Table 1).[25]

In Latin America, large-scale data from Brazil further show these vaccine-related events across different vaccine platforms. A study in Bahia, Brazil, analyzed over 10 million adults who received a first dose between 2021 and 2022 and reported an overall adverse event following immunization. Adverse event prevalence differed by vaccine platform, with higher rates observed following the adenoviral vector vaccine ChAdOx1-S compared to inactivated CoronaVac, while lower rates were reported for mRNA (BNT162b2) and Ad26.COV2.S vaccines (#11 Table 1).[26]

Taken together, there is considerable evidence to suggest a link between vaccination and new-onset MG or exacerbation of existing disease. It appears applicable to all vaccine types and in both men and women. Induction occurs relatively quickly, and antibody levels are elevated. It is well known that antibody production is coordinated by the action of both B and T-cells; it may be deduced that a certain "mechanical trigger" caused expansion of these cells. However, this process is carefully regulated; understanding circumstances where aberrant stimulation occurs may offer insight into how vaccinations may trigger MG.

Immune Response Post Vaccination

The initial immune response to vaccination occurs within minutes to hours, involving dendritic cells, macrophages, and neutrophils. Neutrophils help with rapid antimicrobial activity[27], while monocytes help differentiate into antigen-presenting cells and produce cytokines that influence different adaptive immune responses.[28] Since this review is focused on elevated levels of antibody production, the focus will be more on the adaptive part of the immune response. This happens as B cells begin producing antibodies and T cells expand and differentiate.[29] Both cell types recognize surface features found on viruses, bacteria, or abnormal body cells. Once B cells bind directly to antigens using their B cell receptors (BCRs), they receive help from T helper cells through additional cytokine signals to become fully activated.[30] This process is also known as linked recognition, where B cells and T helper cells recognize

different parts of the same antigen. It also increases B-cell production, memory formation, and so forth. This classical mechanistic pathway ensures that antibody production remains antigen-specific and properly regulated.[31] T cells are activated when antigen-presenting cells (APCs) show that they contain pieces of foreign material, called antigens, on their surface. When a T cell receptor (TCR) recognizes this antigen and receives signals from the immune system, it becomes fully activated and begins to multiply. These activated T cells release cytokines, which are signaling proteins that guide other immune cells to respond to infection. This cell-mediated immunity is essential for the immune system to destroy infected cells and other foreign materials.[32]

Once activated, B cells become plasma cells, which then release antibodies that attach to foreign molecules. A portion becomes memory B cells, which help the immune system respond faster in the future.[33] Similarly, a portion of T cells also become long-term memory cells. These cells will lie dormant but may become activated upon antigen stimulation in the future to coordinate antigen-specific antibody production alongside B cells.[34] After the initial immune activation, both B and T cells can become memory cells, which allows the body to respond more rapidly in the future if it encounters the same antigen. This secondary immune response is stronger and faster than the first response, which is why vaccines are usually really effective in preventing reinfection.[35] In MG, long-lived memory B cells specific to AChR can rapidly reactivate during strong immune stimulation. This amplified response can contribute to temporary symptom worsening when the immune system is heavily triggered. It is not known precisely how these autoreactive memory B cells in MG become activated and amplified, but monitoring this population is key to following disease progression and predicting possible relapse.[36]

Non-specific activation refers to situations where memory B cells are stimulated without antigen-specific binding, and since MG memory B cells persist for long periods, this can boost autoantibody production. This antibody production is a key part of the humoral immune response and provides long-term protection after vaccination.[37] In autoimmune conditions such as MG, autoantibodies against the acetylcholine receptor (AChR) are generally believed to be produced when immune tolerance fails, which most often occurs in adulthood rather than early childhood.[38] This allows B cells that should have been inactivated to begin producing AChR-specific antibodies. Once these autoantibodies are first generated, they can be produced again whenever the immune system is strongly stimulated.[39] T cells can also undergo nonspecific activation. This happens in inflammatory environments by cytokines such as IL-6, IL-1β, and TNF-α. This cytokine-driven T cell activation can amplify immune responses by increasing helper T cell support to B cells, which can indirectly enhance antibody production, and can worsen autoimmunity.[40] Circumstances that commonly reactivate autoantibody production include infections, significant inflammation, vaccination-related immune activation, or physical and emotional stress.[30] Specific factors are outlined below.

Cytokine Storm

Cytokines normally act as messengers that regulate inflammation and direct immune cells to infection sites. However, if cytokine activity becomes excessive and poorly regulated, it can lead to a cytokine storm, which is a dangerous condition where inflammation spreads uncontrollably.[41] This is driven by high levels of IL-6, IL-1β, TNF-α, IFN-γ, and sometimes IL-17, produced mainly by activated monocytes/macrophages and dendritic cells. This can cause tissue damage and may contribute to autoimmune diseases, including Myasthenia Gravis. A cytokine storm creates an intense inflammatory response that stimulates autoreactive B and T cells, which increases the risk of elevated anti-AChR antibody production.[42]

Bystander Activation

Bystander Activation is another example of nonspecific activation of immune cells that occurs due to inflammatory cytokines. Instead of being activated through direct antigen recognition, T and B cells can become activated by cytokines released from nearby immune responses due to these cytokine storms. When activated, these cells may contribute to autoimmune activity by promoting antibody production or inflammatory signaling. In MG, bystander activation is important, as it can increase the likelihood of autoantibody production and can worsen existing symptoms during infections or immune stimulations.[4] Mechanisms for autoimmune activation in MG are not well understood. It remains unclear at which point autoreactive pathogenic memory B cells against AChR are generated via the adaptive immune response. However, these memory B cells persist in the bloodstream. It has been reported that following infections, stress, or hormonal changes, MG symptoms are exacerbated.[13] When someone with a high number of memory B cells goes through strong immune stimulation, such as vaccination, these cells can rapidly reactivate. This increases the potential for additional autoantibody production, which can contribute to worsening symptoms or triggering exacerbations in susceptible individuals. This pattern is supported by clinical data. In MG, rising memory B cell levels are directly associated with a relapse, and have detectable increases in circulating CD27+ memory B cells above ~0.01% of leukocytes, and this can be linked to worsening in most cases.[44]

SARS-CoV-2

The virus responsible for the COVID-19 outbreak, known as SARS-CoV-2, contains a positive-sense, single-stranded RNA genome, which allows the viral RNA to be directly used by the host cell's ribosomes to produce proteins, much like the host's own mRNA.[45] This property enables the virus to rapidly begin replication by making essential nonstructural proteins, such as the RNA-dependent RNA polymerase and viral proteases**,** which are needed for copying the viral genome and processing proteins. The genome also encodes several structural proteins, including the spike (S) protein, which binds to different receptors on the cell surface (e.g., ACE2) and mediates cell entry, and the membrane (M) and envelope (E) proteins, which bind to the viral RNA to form the nucleocapsid.[46] Another important structural feature is its envelope, which is derived from the host cell's lipid membrane and contains the S, M, and E proteins. This envelope

enables the virus to fuse with host cells, making it easier for the virus to enter the cells. COVID-19 vaccines are based on either fragments of the virus structure or inactivated versions of the whole viral particle.

Once infection occurs, this virus targets respiratory and endothelial cells, which trigger inflammation. This causes symptoms such as lung injury, pneumonia, and other organ complications.[47] Common symptoms include fever, cough, fatigue, shortness of breath, and loss of taste or smell. Most mild cases will self-resolve without the need for medical intervention, whilst moderate to severe infections may require antiviral drugs such as Paxlovid alongside other anti-inflammatory therapy.[48] Several of these symptoms overlap significantly with those of MG, most notably respiratory weakness, which is severely compounded by both conditions and could rapidly deteriorate into a medical emergency. Furthermore, COVID-19 infection can cause respiratory inflammation through spike protein-mediated entry into ACE2- expressing cells, which can lead to swelling and reduced oxygen intake, becoming a severe source of respiratory burden for patients.[49]

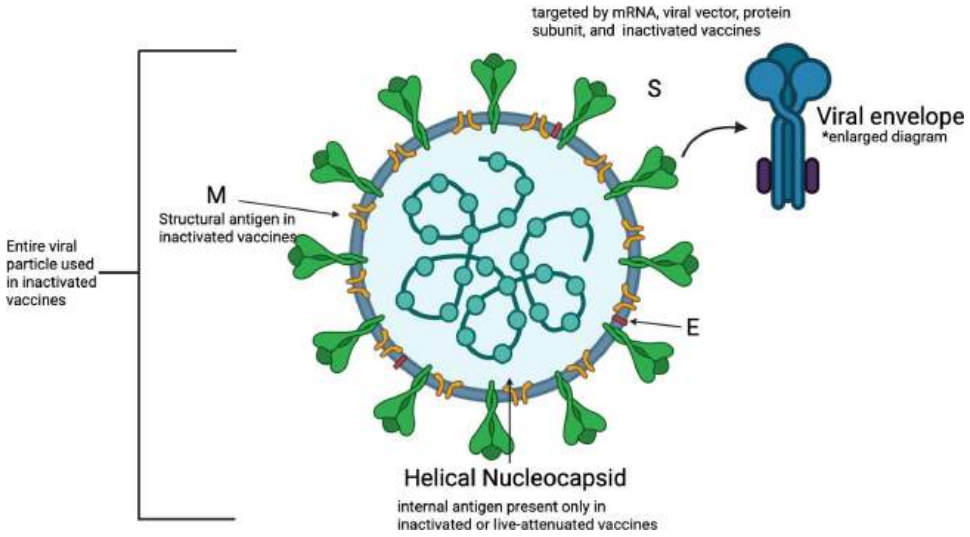


Figure 2. Structure of SARS-CoV-2, an enveloped virus, surrounded by a lipid bilayer derived from the host cell. The surface is studded with glycoproteins (green). This envelope allows viral entry and fusion with host cells.[50] The key components of the virus include the spike (S) protein, the membrane (M), and envelope (E) proteins. The spike (S) protein is the primary antigen targeted by mRNA vaccines, viral vector vaccines, and protein subunit vaccines, which encode or contain only the spike protein. In contrast, inactivated whole-virus vaccines contain multiple viral components, including the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins, leading to broader immune activation. This figure illustrates how vaccine platforms differ in the viral antigens they present, leading to differences in immune activation.

SARS-CoV-2 Components Targeted by Vaccines

The S (spike) protein, found on the surface of SARS-CoV-2, is the primary antigen

targeted across various vaccine platforms. Most vaccines are designed to target multiple regions of this protein: the full-length spike protein, its subunits S1 and S2, the receptor-binding domain (RBD), and the N-terminal domain (NTD) (Figure 2). Targeting these regions and training the immune system via the humoral response ultimately leads to the production of neutralizing antibodies, as well as CD4+ and CD8+ T cells via cell-mediated immunity. These helper T cells assist in coordinating immune responses, promoting B cells' maturation, antibody production, and releasing cytokines—small signaling proteins that regulate and amplify immune activity.[32] Under normal circumstances, the immune system offers protection against foreign antigens. However, dysregulated activation of B and T cell populations could lead to adverse events caused by dysregulated antibody production. Additionally, hyperactive T helper cells can amplify these aberrant responses and further exacerbate the amplification of B cells.[51] Regulatory T cells (Treg) contribute to the regulation of B and T cell proliferation. However, in several autoimmune disorders, the regulatory function of Treg cells is often impaired, further adding to the immune imbalance and autoantibody production against self-antigens.[35] Worth noting is that in the context of vaccination, a brief period of increased immune activity is normal, but in situations where amplification of immune cells becomes sustained, development of autoimmune-attributed pathophysiology occurs.

COVID-19 Vaccines and Their Impact on Autoimmune Diseases

To explain these findings, several mechanisms have been proposed. One of the leading theories is molecular mimicry, where components of the vaccine resemble certain human proteins. This resemblance may lead to immune cross-reactivity, where the immune system mistakenly attacks the body's own tissues. Research by Kanduc *et al*. supported this hypothesis by identifying shared heptapeptides between SARS-CoV-2's spike glycoprotein and various human proteins. As a result, antibodies generated after vaccination could bind to host proteins, potentially triggering autoimmune responses.[23]

Another proposed mechanism involves adjuvants. Adjuvants can act as Toll-like receptor (TLR) ligands, activating innate immune cells and increasing the effectiveness of the vaccine. However, some adjuvants may provoke immune-related disorders. The body is capable of producing effective antibody responses through the adaptive immune system, even without the use of these adjuvants. Henceforth, while they can be helpful, adjuvants may also contribute to unintended immune activation.[52] Since adjuvants enhance immune activation through TLR signaling, they also intensify cytokine production and germinal center formation, which directly promotes the increase of B cell and antibody generation. In MG, this stronger stimulus can activate autoreactive memory B cells, increasing the risk of elevated autoantibody production.

A third proposed mechanism is bystander activation, which refers to the non-specific activation of T and B cells through cytokines instead of direct receptor engagement. In this process, immune cells, including autoreactive T or B cells, might become activated by cytokines released during inflammation. Once activated, these autoreactive cells may then be drawn to inflammation sites where they can initiate and trigger autoimmune responses.[40]

These same processes can also occur during the COVID-19 infection itself. SARS-CoV-2 includes much higher levels of inflammatory cytokines, including IL-6, IL-1β, and TNF-α. The virus triggered these more than vaccines. This is because active viral replication produces continuous PAMP signals, driving prolonged cytokine release and widespread immune cell recruitment. This intense inflammation may lead to non-specific T and B cell activation becoming more likely, which increases the risk that autoreactive memory B cells in MG patients become activated and produce additional autoantibodies. These effects can worsen existing autoimmune diseases or even trigger new onset autoimmunity in susceptible patients.[53]

Direct B-cell Activation by Viruses

The risk to MG patients is not only limited to vaccines but also extends to full viral infections as well. Viruses can activate B cells through both antigen-specific and non-specific pathways in a T-cell-independent manner. First, BCR cross-linking happens when repetitive viral surface proteins (such as envelope or capsid structures) cluster multiple B cell receptors at the same time, which triggers the activation of these cells.[53] This is called crosslinking. Second, viral components can be detected through pattern recognition receptors (PRRS), including TLR7, TLR9, and RIG-I, which signal to promote cytokine release and broad B cell activation, creating an inflammatory cascade. This refers to PAMP recognition.[54] The virus triggers the immune system through pathogen-associated molecular patterns (PAMPS) found in the RNA. Single-stranded RNA and the double-stranded RNA formed during replication can be detected by immune receptors, including RIG-I, MDA5, and TLR7/8. These activate cytokines to initiate antiviral defense (producing B cells against the target, etc.). Because this signaling creates a strong inflammatory state, it can also stimulate nearby memory B cells in a non-specific way, increasing the chance of additional autoantibody production in MG. Finally, certain viral proteins can function as B cell mitogens, directly driving polyclonal B cell proliferation and enhancing the activation of memory B cells, including autoreactive ones.[55] For the SARS-CoV-2 virus, this includes N, S, and accessory proteins. Surface antigens (capsid, envelope). Antigen variation and presentation. The virus Spike protein is the main surface antigen and the primary target. It has a glycan shield, which can partially hide antigenic sites, and can change how strongly antibodies bind and how much stimulation is required to pass the immune activation threshold. Other structural components, including the membrane, envelope, and nucleocapsid proteins, can also contribute to how efficiently the virus can enter cells and how much it can be detected by the immune systems.[56]

Types of Vaccinations

Vaccine platforms activate the immune system using different components of a virus. Therefore, assessing the pattern of immune response triggered by each type of vaccine may tentatively allow an assessment of MG severity in susceptible individuals.[57]

mRNA Vaccines

mRNA vaccines deliver genetic instructions for the SARS-CoV-2 spike protein directly into host cells using lipid nanoparticles. Once inside the cell, the mRNA is translated by ribosomes to produce spike proteins, which then trigger both humoral and cellular immune responses.[58] The newly made spike proteins then activate cytotoxic T cells, or CD8+ cells, and can help activate B cells, increasing antibody production and sustaining immunity. Importantly, the mRNA never enters the cell's nucleus, which eliminates the risk of the genetic material coding for the spike protein being integrated into the human genome. Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) are two prominent examples of mRNA-based vaccines.[59] In most mRNA vaccines, while both T and B cells are strongly activated, studies have shown that they cause particularly high B cell responses compared to other vaccine types. This strong response occurs because mRNA vaccines produce high amounts of spike protein inside the host cells, which can lead to prolonged antigen presentation. This stimulation can generate both robust CD4+ T cell recruitment and strong B cell activation, which together create very high antibody titers.[60] For MG patients, this strong B-cell stimulation increases the risk of symptoms worsening, because MG immune responses primarily depend on autoantibody production. Furthermore, mRNA molecules can also be sensed by innate immune receptors such as TLR7, TLR8, and RIG-I, which generate a brief inflammatory response, and this cytokine-rich environment can indirectly broaden B cell activation. This allows pre-existing memory B cells, including autoreactive ones, to become activated in a bystander, non-specific way. In MG, this provides one possible explanation for why a small portion of patients can experience symptoms worsening after mRNA vaccination.[61]

Inactivated Virus Vaccines

Inactivated vaccines are structurally intact but have been chemically or thermally inactivated[62], meaning that they cannot replicate or cause disease. Numerous antigen components may trigger immune activation. This vaccine requires the use of adjuvants, which are compounds added to enhance immune responses.[63] Adjuvants function by activating innate immune sensors, such as Toll-like receptors (TLRs) and inflammasome pathways that increase antigen uptake, promote cytokine release, and stimulate the maturation of dendritic cells. However, these signals further strengthen both B and T cell activation, which is not favorable in MG patients. Furthermore, enhancement of responses is not antigen specific, resulting in T and B cells activation regardless of their specificity.[64] For healthy individuals, this can improve vaccine efficacy, but in susceptible individuals, there is an enhanced risk of autoreactive antibody production by B cells.[65] In MG patients, it poses a higher risk of disease initiation or recurrence.

Viral Vector Vaccines

Viral vector vaccines use modified, non-replicating viruses, such as adenovirus, to deliver the gene encoding the SARS-CoV-2 spike protein into host cells.[66] After entering the cell, the viral vector releases its DNA into the nucleus, where the spike gene is transcribed into mRNA and then translated in the cytoplasm into spike protein. This antigen is then displayed on the cell

surface and recognized by the immune system, generating CD4+ and CD8+ T cell responses, as well as antibody production.[67]

Unlike inactivated vaccines, viral vector vaccines do not require adjuvants because the viral capsid itself provides an innate immune stimulus through pattern recognition receptors. Viral vectors can persist for longer periods of time in the body than mRNA, hence antigen presentation may occur for a more extended period, potentially increasing the duration and magnitude of the immune response.[66] Additionally, viral vector vaccines tend to induce stronger T cell responses compared to B cell antibody titers because adenoviral vectors efficiently activate antigen-presenting cells and stimulate both CD4 + helper T cells and CD8+ cytotoxic T cells. Since these T cell subsets coordinate defense mechanisms rather than producing antibodies, they contribute less directly to MG-related autoantibody increases. In contrast to inactivated vaccines, vector vaccines generate less nonspecific inflammation, meaning there is less bystander activation of autoreactive memory B cells. Compared with mRNA vaccines, they also produce lower peak antibody titers. This makes viral vector vaccines potentially safer for MG patients.[68]

Other Vaccines

Other vaccine types, though less prevalent globally, include live-attenuated vaccines (LAV) and Protein subunit vaccines. LAV uses weakened SARS-CoV-2 that can replicate without causing severe illness, closely mimicking natural infection and generating broad immune responses. These vaccines could stimulate both B and T cells robustly, but their use in autoimmune patients is limited due to the potential for uncontrolled immune activation.[69] Examples include Codagenix and Meissa Vaccines. Protein subunit vaccines, such as Novavax, contain purified spike protein fragments that cannot cause infection, but with the use of adjuvants can stimulate antibody production and T cell responses.[70] Since these vaccines rely heavily on B-cell stimulation, MG patients could theoretically experience greater immune sensitivity compared to other vaccines, like vector vaccines. However, reported cases remain rare.[71] Across these vaccine types, adjuvant use and strong innate activation can also stimulate B cells in a non-specific way. For MG patients, this activation can increase the likelihood of autoreactive memory B cells becoming active.

Conclusions

This literature review highlights the risks when immune responses are aberrant and become dysfunctional. Clinical reports appear to suggest a link between MG initiation or flare-ups, highlighted by an increase in autoantibody production in affected individuals. Based on clinical reports and known theories such as molecular mimicry, bystander activation, and immune modulation, we propose non-specific B and T cell activation as a plausible explanation. Furthermore, we review how different structural components of the SARS-CoV-2 virus may elicit stronger immune responses. Whilst actual comparative data is limited, it appears that inactivated vaccines triggered more newly developed cases of MG, although there is a risk associated with all vaccine types. Taken together, clinical findings suggest that COVID-19

vaccines are generally safe, but there is a small subset of patients who are particularly vulnerable. A key limitation in the current literature is the lack of research data into the disease-initiating mechanisms of MG and other autoimmune diseases. Additional clarity is also required on the triggering factor of disease initiation and symptomatic development. Furthermore, it remains unclear why certain individuals never develop autoimmune complications even though they may be predisposed to doing so. This is likely attributable to a combination of genetic predisposition, preexisting autoreactive memory B and T cell populations, environmental factors, or a combination of all of these.

Based on the patterns identified throughout this review, vaccine safety needs to be carefully evaluated, especially in susceptible individuals. This is especially relevant to other autoimmune diseases with even more severe clinical presentation than MG, where even a mild relapse may become fatal. In most patients, autoantibody levels are typically raised. If vaccination is deemed to be necessary, administration when autoantibody levels are at the lowest point would minimize the risk of autoimmune initiation or relapse. Further research detailing autoantibody production triggering potential posed by different vaccine types may also further enhance overall safety. By continuing to study both immunological mechanisms and real-world patient outcomes, clinicians and researchers may be able to propose safer health strategies that will further enhance the safety of vaccines in susceptible individuals with autoimmune disease.

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How Does Pupil Diameter Change Over Time After Sudden Exposure to Light?
By Noella Gu

1. Introduction

The pupil is the dark circular opening in the centre of the eye that controls how much light enters. When the environment becomes bright, the pupil becomes smaller to limit the amount of light reaching the retina; when the environment becomes darker, the pupil becomes larger to allow more light to enter. This automatic response is called the pupillary light reflex, and it is controlled by the nervous system [1].

This research began with a simple question: how does pupil diameter change over time after sudden exposure to light? At first the answer may seem obvious, in that the pupil gets smaller. However, the change does not happen instantly. After light exposure there is a short delay before the pupil begins to react. The pupil then constricts rapidly, reaches a minimum diameter, and gradually recovers. Pupil size is therefore not a fixed value but a continuously changing measurement over time.

The pupillary light reflex is clinically important because it gives information about how the eye, brain and nervous system are functioning. In clinical settings, pupil responses are measured using pupillometry, which records pupil diameter as a time series. Researchers use measurements such as baseline diameter, constriction latency, constriction amplitude, constriction velocity and recovery time to describe the shape of the response [2]. Because these measurements change over seconds, the pupil response is a strong subject for mathematical modelling.

The topic also connects to physics. Light intensity affects how much the pupil constricts, and the pupil itself acts as an aperture controlling the amount of light entering the eye. A sudden increase in light produces a biological response, but that response can be studied through a graph of pupil diameter against time. This investigation therefore connects optics, biology, medicine and mathematics.

In this paper a mathematical model is developed to describe the change in pupil diameter after sudden light exposure. A simple exponential decay function is not sufficient, because pupil diameter does not decrease forever. The pupil starts at a normal size, decreases quickly after the stimulus, reaches a minimum, and then moves back toward its original size. The model must represent both a rapid constriction phase and a slower recovery phase.

The model uses time after light exposure as the independent variable and pupil diameter as the dependent variable. The purpose of this investigation is not to diagnose an eye or neurological condition. The goal is to use a continuous mathematical model to understand the general shape of the pupillary light reflex, and to explain why pupil diameter changes in a curved pattern rather than a sudden jump.

2. Understanding the Mathematical Models

To model how pupil diameter changes after a sudden light stimulus, it is necessary to keep two processes in mind. The pupillary light reflex describes the constriction and subsequent dilation of the pupil in response to light, and these two movements are produced by the antagonistic actions of two muscles in the iris [3]. The iris sphincter muscle, innervated by the parasympathetic nervous system, contracts to constrict the pupil, while the iris dilator muscle, innervated by the sympathetic nervous system, contracts to dilate it [2]. Because two different pathways govern the two halves of the response, there is no reason to expect the constriction and the recovery to occur at the same speed. The mathematical model must therefore describe a response that first decreases and then increases again, and it should allow the two phases to differ in duration.

2.1 The latency shift

The pupillary light reflex does not begin at the exact moment the light is presented. There is a short delay, called the latency period, during which the neural signal travels from the retina to the iris muscles. During this period the pupil remains close to its starting diameter.

To include this delay, a shifted time variable is used:

$\tau = t - L$

where

τ = time measured from the start of the main response

t = time after the sudden light exposure

L = latency period

For the healthy control values used in this paper, L = 0.2085 s, taken from the pupillometry measurements of 134 healthy adolescent control participants reported by Master and colleagues [4]. The model is defined piecewise: for $t < L$ the diameter remains at its maximum value, and for $t \geq L$ the response function applies. Using τ rather than t means that every subsequent formula describes the shape of the response only, without carrying the delay through every calculation.

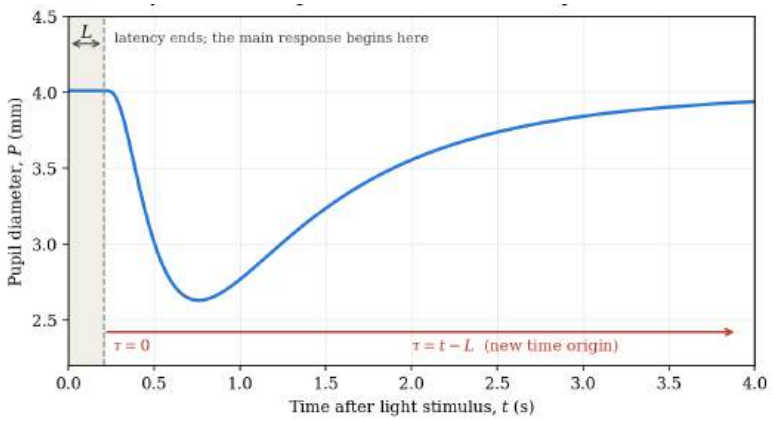


Figure 1. Latency shift allows for the measurement of time from the start of the pupil response (e.g. 0.2085 s), not the stimulus

2.2 The general structure: a baseline minus a pulse

Because the pupil starts at its maximum diameter, falls, and returns toward that same maximum, the response is naturally written as a constant minus a single pulse:

$P(t) = D_{max} - A \cdot g(\tau)$

where

$P(t)$ = predicted pupil diameter at time t

D_{max} = resting (maximum) pupil diameter

A = constriction amplitude, $A = D_{max} - D_{min}$

$g(\tau)$ = a shape function

For this structure to be correct, g must satisfy three conditions:

1. $g(0) = 0$, so the diameter equals D_{max} at the moment the response begins;
2. g rises to a single maximum value of 1, so the diameter reaches exactly $D_{min} = D_{max} - A$;
3. $g \to 0$ as $\tau \to \infty$, so the pupil returns to its resting diameter.

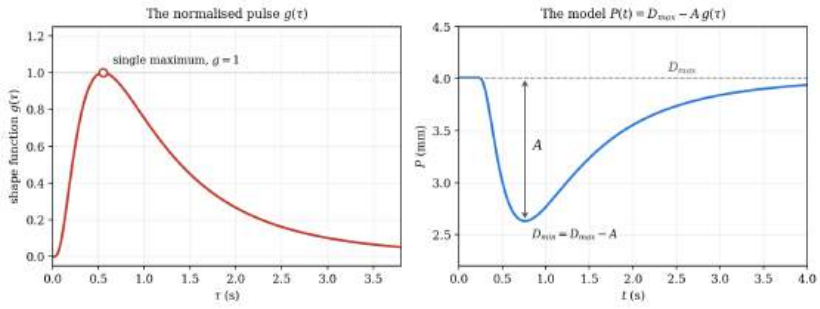


Figure 2. The normalised pulse g(τ) (left) and the model P(t) = D_{max} − A·g(τ) obtained by subtracting it from the resting diameter (right).

The whole modelling problem therefore reduces to a single question: which parent function produces an asymmetric one-sided pulse satisfying these three conditions?
Previous quantitative work on pupil responses has used the gamma family of functions for this purpose. Hoeks and Levelt modelled the pupillary response to a brief stimulus as an Erlang gamma impulse response with two free parameters, and this function has since been used widely as a standard pupil response function [5]. The gamma function is one-sided and skewed, so it satisfies the three conditions above. It is not, however, the only function that does so, and the alternative developed in this paper is constructed from a more familiar parent function.

2.3 The Gaussian pulse and the problem of symmetry

A natural candidate for a pulse is the Gaussian, or bell-shaped, function:

$g(x) = e^{-x^2 \div 2}$

This function has exactly one maximum, at $x = 0$, and decays to zero in both directions. It satisfies conditions 2 and 3 immediately. However, two features make it unsuitable in its raw form. First, it is defined for all real x, including negative values, whereas the pupil response only exists for $\tau > 0$. Second, and more importantly, it is perfectly symmetric about its maximum.

The pupillary light reflex is not symmetric. In the reported control values the pupil completes its constriction in roughly half a second, while the recovery toward the resting diameter takes several times longer [4]. This is consistent with the two phases being driven by different autonomic pathways [3]. Any symmetric function must therefore misrepresent one phase or the other.

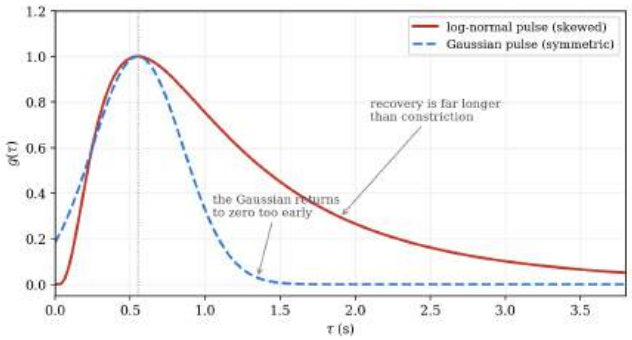


Figure 3. A symmetric Gaussian bell cannot match the response with a long recovery tail

2.4 The log-normal substitution

Both problems with the Gaussian are solved by a single substitution. Replace the variable x by the logarithm of scaled time:

$x \longrightarrow \ln(\tau \div \tau_m) \div s$

Because the natural logarithm maps the interval $(0, \infty)$ onto the whole real line $(-\infty, \infty)$, the resulting function is automatically restricted to positive times, and it decays to zero at both ends of that interval. Substituting gives

$g(\tau) = \exp[\ -(\ln(\tau \div \tau_m))^2 \div (2s^2)\ ]$

and the complete model becomes

$P(t) = D_{max} - A \cdot \exp[\ -(\ln(\tau \div \tau_m))^2 \div (2s^2)\ ], \quad \tau = t - L$

This is referred to as the log-normal pulse model. It has three parameters, each with a direct physical meaning:

A = constriction amplitude, so that $D_{min} = D_{max} - A$

τ_m = time from the end of latency to the minimum diameter

s = shape parameter controlling the asymmetry of the response

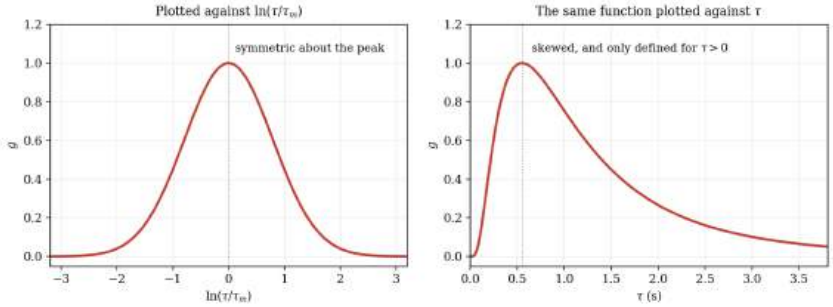


Figure 4. The same function plotted against $\ln(\frac{\tau}{\tau_m})$, where it is symmetric (left), and against τ, where it becomes one-sided and right-skewed (right).

2.5 The role of each parameter

The parameter A controls the depth of the constriction without affecting its timing. Increasing A produces a deeper minimum while the turning point remains at the same instant.

The parameter τ_m controls when the minimum occurs. Increasing τ_m shifts the entire pulse later in time and also stretches it, because the substitution acts multiplicatively on time rather than additively.

The parameter s controls the width and the asymmetry. A small value of s produces a narrow, nearly symmetric pulse; a larger value produces a broad pulse with a long recovery tail.

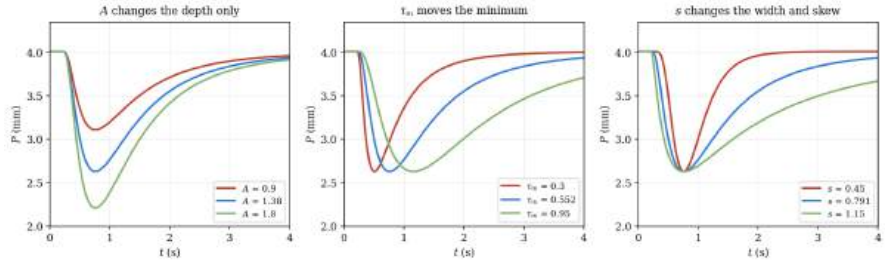


Figure 5. The effect of the three parameters: *A* controls the depth, τ_m the timing, and *s* the width and asymmetry of the modelled response.

2.6 Locating the minimum diameter

The time of maximum constriction is found by differentiating the shape function. Using the chain rule,

$g'(\tau) = -g(\tau) \cdot \ln(\tau \div \tau_m) \div (s^2\ \tau)$

Since $g(\tau) > 0$ for all $\tau > 0$, and $s^2\ \tau > 0$, the derivative is zero only when $\ln(\tau \div \tau_m) = 0$, that is, when $\tau = \tau_m$. Substituting $\tau = \tau_m$ gives $g(\tau_m) = e^0 = 1$, so the minimum diameter is

$P_{min} = D_{max} - A$

The model therefore has exactly one turning point for every possible choice of parameters, and both the location and the depth of that turning point are given directly by the parameters rather than requiring numerical search.

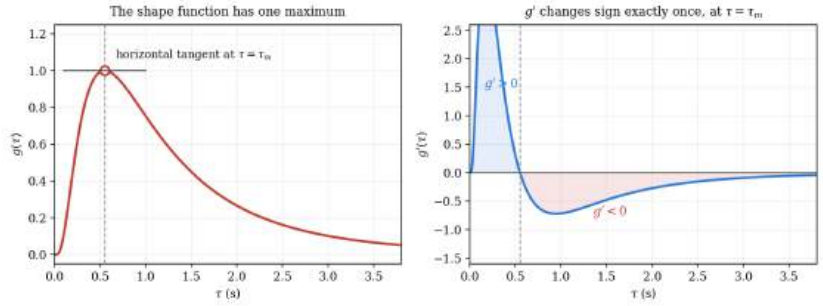


Figure 6. The shape function has a horizontal tangent at $\tau = \tau_m$ (left), and its derivative changes sign exactly once (right), so the model has exactly one turning point.

3. Main Body

In this section the pupil response is modelled using values reconstructed from published pupillary light reflex data. The purpose is not to choose a final function immediately, but to compare several possible parent functions and decide which one best represents the shape of the pupil-diameter graph.

The values used are pupillary light reflex metrics measured in healthy controls using automated dynamic infrared pupillometry [4]. For the healthy control group, the reported mean values were as follows.

Table 1. Published pupillary light reflex metrics for the healthy control group (n = 134) [4]

Measurement	Published mean value
Maximum pupil diameter	4.01 mm
Minimum pupil diameter	2.63 mm
Percentage constriction	33.66%
Latency	208.50 ms
Average constriction velocity	2.50 mm/s
Peak constriction velocity	3.91 mm/s
Average dilation velocity	1.22 mm/s
Peak dilation velocity	1.64 mm/s
T75 recovery time	1.51 s

3.1 Reconstructing anchor points from the published metrics

The maximum pupil diameter was reported as 4.01 mm with a 99.44% confidence interval from 3.82 mm to 4.21 mm [4]. The value 4.01 mm can therefore be used as the mean starting value, while the interval shows a reasonable range for the true population mean. The confidence interval describes the precision of the group average and not the spread of individual participants.

The amount of constriction is

$A = 4.01 - 2.63 = 1.38$ mm

The reported latency of 208.50 ms gives $L = 0.2085$ s, confirming that the response does not begin instantly. Using the average constriction velocity, the time required to move from the maximum to the minimum diameter is

Time for constriction $= 1.38 \div 2.50 = 0.552$ s

so the minimum diameter is reached at

$0.2085 + 0.552 = 0.7605$ s

The study also reports a T75 recovery time of 1.51 s, the time for the pupil to redilate from its minimum diameter to 75% of its constriction amplitude [4]. The 75% recovery diameter is

$2.63 + 0.75(4.01 - 2.63) = 2.63 + 0.75(1.38) = 3.665$ mm

and the time of this recovery point is $0.7605 + 1.51 = 2.2705$ s

Table 2. Reconstructed pupil-diameter anchor points used for the mathematical model

Time t (s)	Pupil diameter P(t) (mm)	Source of value
0	4.01	Published maximum pupil diameter [4]
0.2085	4.01	Published latency value [4]
0.7605	2.63	Published minimum diameter; time estimated from average constriction velocity [4]
2.2705	3.665	75% recovery point estimated from published T75 [4]

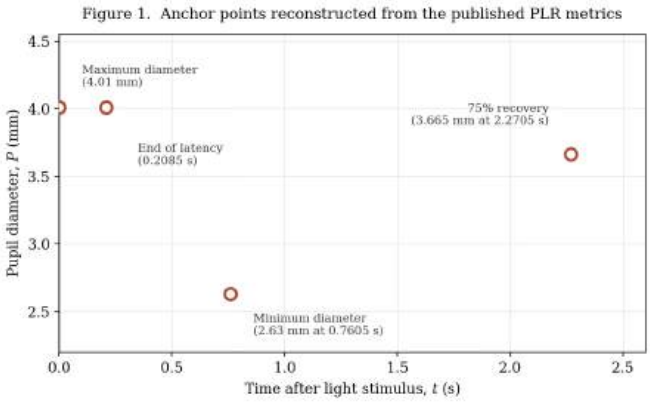


Figure 7. The four anchor points reconstructed from the published pupillary light reflex metrics. These four values, rather than a raw recording, are the starting point of the investigation.

These four values are not a raw time series. They are reconstructed anchor points derived from published summary measurements, and they capture the most important features of the response: initial diameter, delay, minimum diameter and recovery. Interpolating between them produces a continuous curve for the first 2.5 seconds.

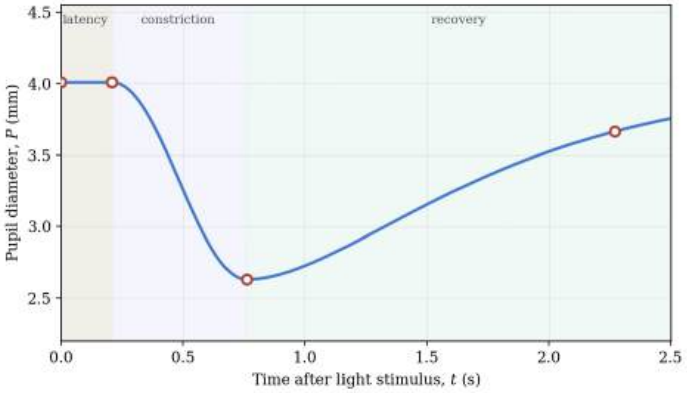


Figure 8. Reconstructed pupil-diameter response from 0 s to 2.5 s, showing the latency period, the constriction phase, the minimum diameter and the recovery phase.

Extending the reconstruction to six seconds produces the data set against which every candidate model is tested. Sampling at intervals of 0.01 s gives 601 points.

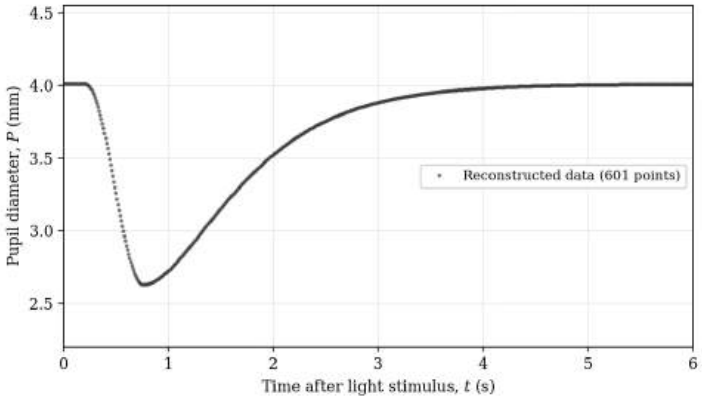


Figure 9. The reconstructed pupil-diameter data used for model fitting, sampled every 0.01 s from 0 s to 6 s.

3.2 Assumptions

Assumption 1. The pupil begins at a maximum diameter of approximately 4.01 mm.
Assumption 2. The pupil does not constrict instantly after the light stimulus; the delay is 0.2085 s.
Assumption 3. The pupil reaches a minimum diameter of approximately 2.63 mm.
Assumption 4. The pupil begins to recover after reaching its minimum diameter.
Assumption 5. Pupil diameter can be represented as a continuous function of time.
Assumption 6. The model is based on published group averages, not on one individual.
Assumption 7. Other factors, such as tiredness, emotion, blinking, medication and individual differences, are not included.

3.3 The linear model

The first model tested is a linear model, which has the form

$P_1(t) = mt + b$

where m is a constant rate of change and b is the initial pupil diameter. A linear model assumes that pupil diameter changes by the same amount every second. Fitting to the reconstructed data gives

$P_1(t) = 3.178 + 0.1704\ t$

This model is too simple. The pupil stays near its starting diameter during latency, then constricts quickly, and later recovers. A straight line has no turning point and so cannot represent latency, rapid constriction, minimum diameter and recovery together. The fitted gradient is in fact positive, because over a six-second window the pupil spends most of its time recovering.

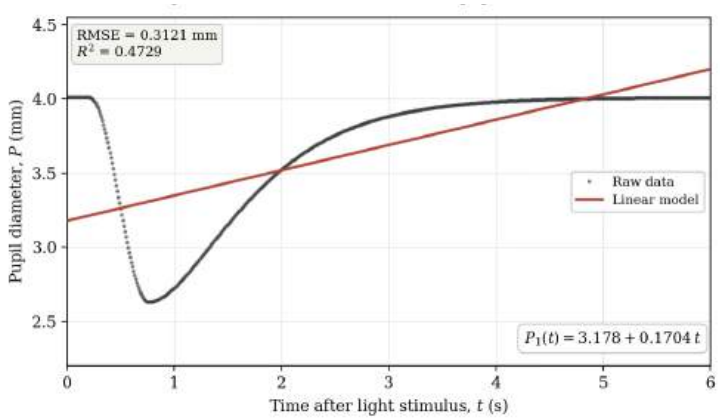


Figure 10. A linear model, representing pupil diameter as changing at a constant rate over time.

3.4 The exponential model

The second model tested is an exponential model, which has the form

$P_2(t) = S + Ae^{-k\tau}$

where S is the stable diameter after adjustment, A is the difference between the starting and stable diameters, k is the rate of approach, and τ is time after latency. An exponential model is better than a linear model because it can show a fast early change followed by slower stabilisation, which matches the constriction phase. Constraining the curve to begin at 4.01 mm and approach 2.63 mm, and fitting only the rate over $t \leq 0.80$ s, gives

$P_2(t) = 2.63 + 1.38\ e^{-3.0608\,\tau}$

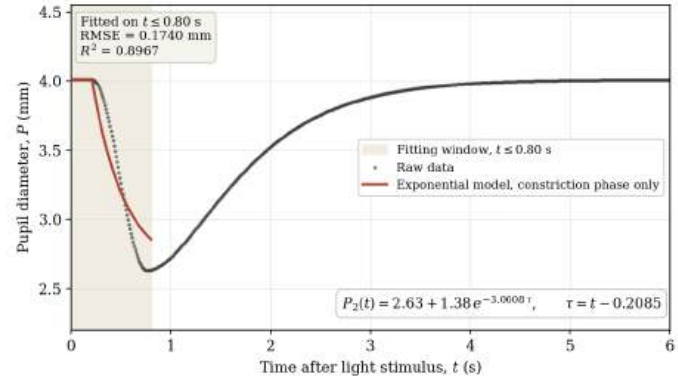


Figure 11. An exponential model restricted to the constriction phase. The fit is reasonable but not exact, because the exponential is steepest at the instant the response begins whereas the measured constriction accelerates gradually.

A simple exponential decay function of the form $S + A \cdot \exp(-k\tau)$ can only move toward the single value S.

If the pupil simply became smaller forever, this function would be sufficient. However, the pupil does not continue shrinking. It constricts, reaches a minimum diameter, and then begins to recover toward its original size. A monotonic decreasing function has no turning point, so it cannot represent a response that reverses direction. The model must describe both constriction and recovery within a single continuous expression.

Fitted over the whole six-second record instead, the exponential collapses. The optimiser drives the decaying term almost to zero and returns an almost horizontal line, because no monotonic function can follow a curve that falls and then rises.

$P_3(t) = 3.677 + 0.5495\ e^{-7.690\ t}$

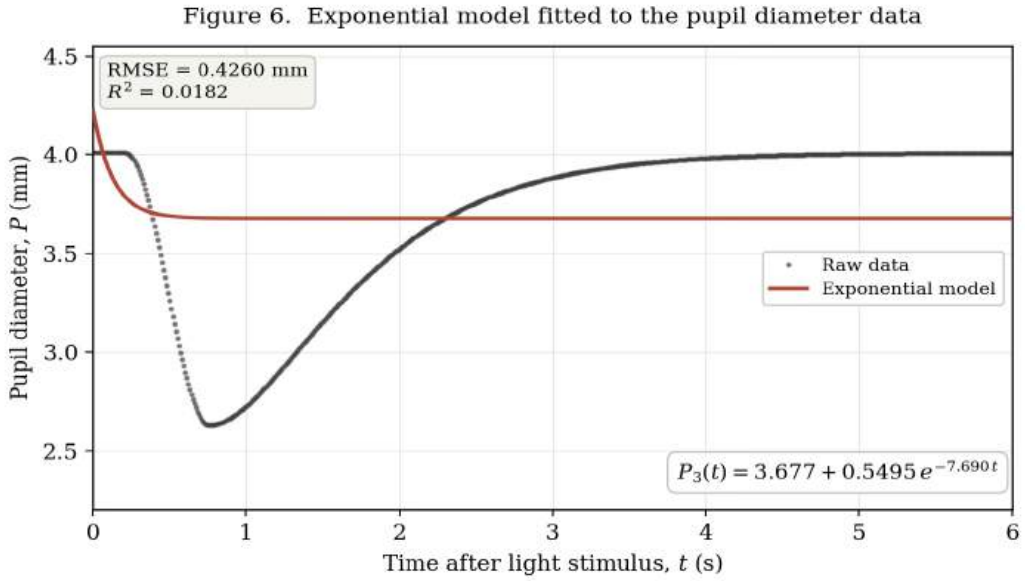


Figure 12. An exponential model fitted over the full record. Exponential functions decrease continuously and never turn upward, so they cannot represent the recovery phase of the pupillary light reflex.

This establishes the need for a model with a turning point.

3.5 The polynomial models

The third model tested is a polynomial model. A cubic polynomial has the form

$P_4(t) = at^3 + bt^2 + ct + d$

where a, b, c and d are constants chosen to fit the data. Fitting to the reconstructed data gives

$P_4(t) = -0.04504\ t^3 + 0.39912\ t^2 - 0.76433\ t + 3.62480$

A polynomial can bend and turn, so it follows the data more closely than a straight line or a single exponential. Its weakness is that the constants have no clear physical meaning: the values above do not represent latency, constriction speed, stable pupil size or recovery. Differentiating gives turning points at t = 1.202 s and t = 4.706 s, so the cubic bottoms out 0.44 s too late and 0.57 mm too shallow, and then rises above the resting diameter to 4.17 mm before turning down again. A cubic has two turning points whenever it has any, but the physiology has exactly one reversal.

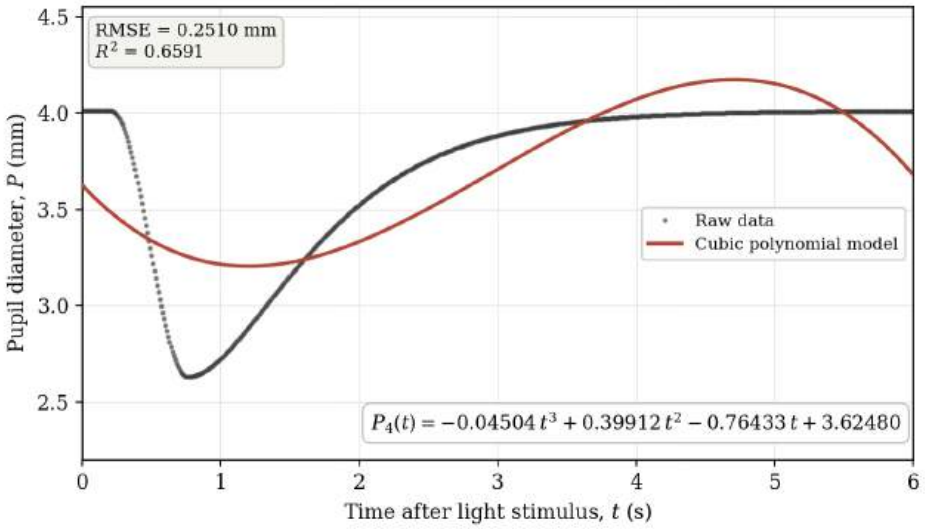


Figure 13. A cubic polynomial model. It captures the turning point more flexibly than an exponential decay model, but its second turning point is an artefact of the parent function rather than a feature of the response.

Increasing the degree improves the fit further. A degree-six polynomial reduces the error substantially, but the improvement is bought with seven coefficients rather than with any physical insight, and the curve develops a spurious bump near t = 5.5 s before turning downward at the end of the window. It also predicts 4.58 mm at t = 0, above the measured resting diameter.

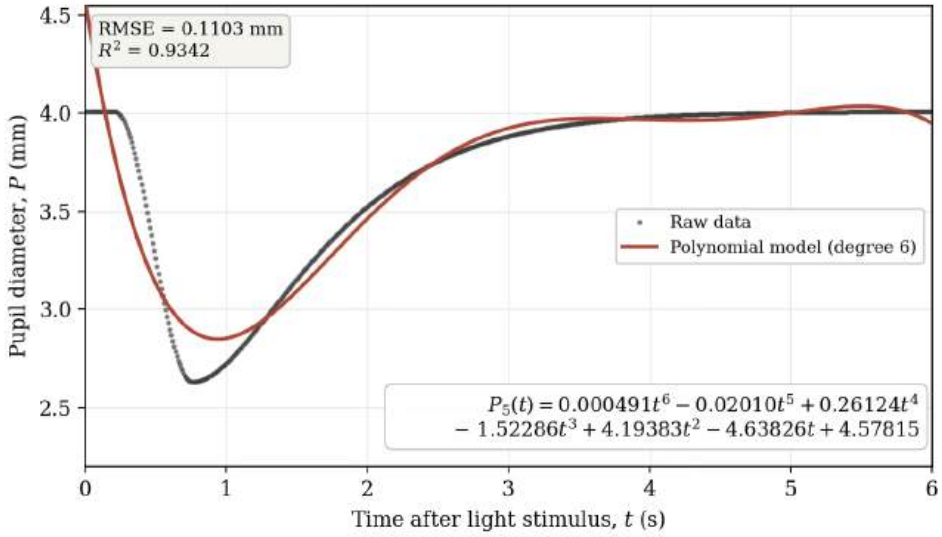


Figure 14. A degree-six polynomial model. Adding terms makes the curve follow the data more closely, but the model becomes unrealistic near and beyond the edges of the measured range, which is a form of overfitting.

3.6 The damped sine model

The fourth model tested is a damped sine model, which has the form

$P_5(t) = P_0 - Ae^{-k\tau} \sin(\omega\tau)$

where P_0 is the initial diameter, A is the size of the response, k is a damping constant, ω is the frequency of adjustment and $\tau = t - 0.2085$. This model combines two ideas: the exponential

term makes the response diminish over time, while the sine term allows the curve to fall and then return upward. At the moment the main response begins, $\tau = 0$, so $e^0 = 1$ and $\sin(0) = 0$, giving $P(t) = P_0$. The model therefore starts exactly at the resting diameter, which matches the idea of latency. Fitting to the reconstructed data gives

$P_5(t) = 4.01 - 5.6035\, e^{-1.3198\tau} \sin(0.8339\tau)$

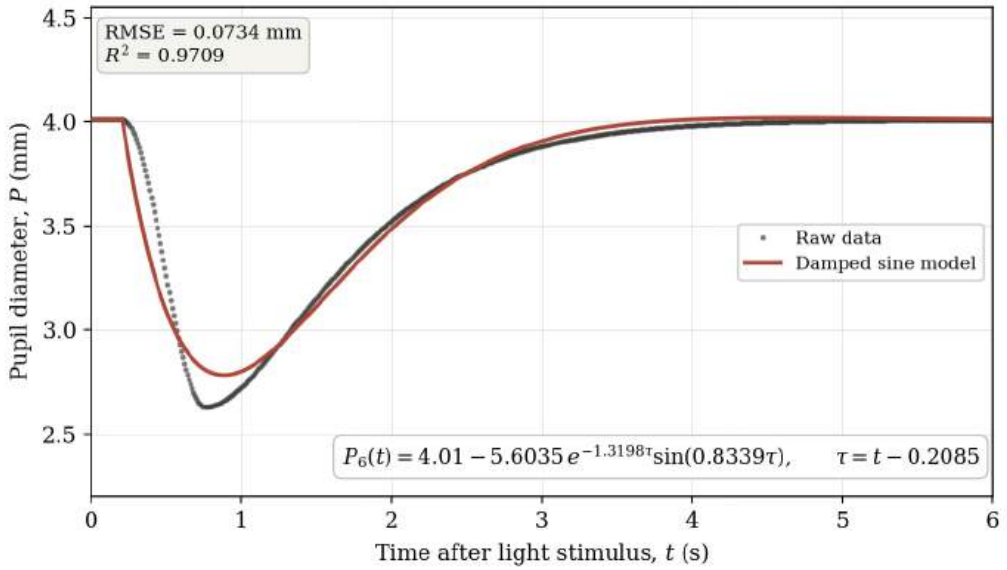


Figure 15. A damped sine model, representing constriction and recovery but capable of producing repeated oscillations.

The fit is good, but two features are troubling. The fitted amplitude of 5.60 mm is larger than the pupil itself, which shows the parameters are absorbing shape error rather than carrying meaning. More fundamentally, the sine function is periodic: extending the model beyond the fitted window predicts a second constriction that the pupillary light reflex does not produce. The damped sine model is therefore useful for comparison, but another parent function is more appropriate.

3.7 The log-normal pulse model

The fifth model tested is the log-normal pulse model derived in Section 2.4:

$P_6(t) = P_0 - A \cdot \exp[\, -(\ln(\tau \div \tau\square))^2 \div (2s^2)\,]$

Unlike the previous models, its parameters are not obtained by curve fitting. They are read directly from the published metrics. The initial diameter is

$P_0 = 4.01$

the constriction amplitude is

$A = 4.01 - 2.63 = 1.38$

and the time from latency to minimum diameter is

$\tau_m = 0.7605 - 0.2085 = 0.552$

The remaining parameter s is fixed by requiring the model to pass through the 75% recovery anchor, that is, P(2.2705) = 3.665. Solving this condition gives s ≈ 0.791. The complete model is therefore

$P_6(t) = 4.01 - 1.38 \cdot \exp[\, -(\ln((t - 0.2085) \div 0.552))^2 \div (2(0.791)^2)\,], \quad t > 0.2085$

$P_6(t) = 4.01, \quad 0 \le t \le 0.2085$

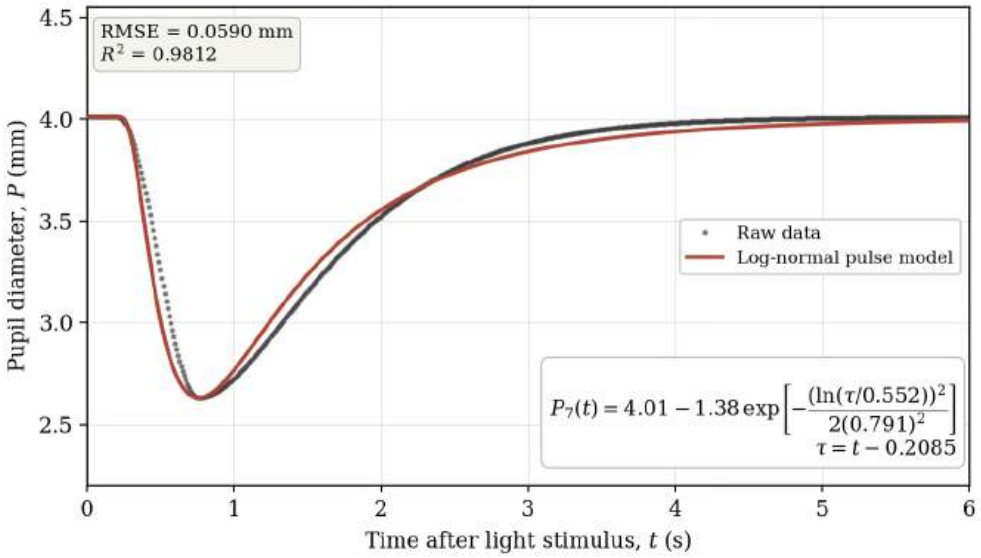


Figure 16. The log-normal pulse model, representing one main constriction response followed by recovery without repeated oscillations. Its parameters are derived from the published metrics rather than fitted to the curve.

The model produces one main constriction pulse: the response grows, reaches a single strongest point, and then fades. Unlike the damped sine model it cannot create repeated oscillations, and unlike the polynomial models every constant carries a physical meaning.

3.8 Comparing the models

To compare the models fairly, the error between the data and each model is calculated at every time point:

Error = actual pupil diameter − predicted pupil diameter

and summarised using the root mean square error:

RMSE = √((sum of squared errors) ÷ n)

The choice of n matters. If the error is evaluated at only the four anchor points, every model that is constructed to pass through those anchors returns an RMSE of approximately zero, which measures the construction rather than the fit. All errors reported here are therefore evaluated against the full reconstructed series of n = 601 points shown in Figure 9. The coefficient of determination R^2 is also given, where a value of 1 indicates a perfect fit and a value of 0 indicates a model no better than predicting the mean.

Table 3. Comparison of the tested mathematical models, evaluated on 601 data points from 0 s to 6 s, except the constriction-only exponential, which is evaluated on its own fitting window ($t \leq 0.80$ s)

Model	Equation	RMSE (mm)	R^2
Linear	$P_1(t) = 3.178 + 0.1704t$	0.3121	0.4729
Exponential (constriction only)	$P_2(t) = 2.63 + 1.38e^{(-3.0608\tau)}$	0.1740	0.8967
Exponential (full record)	$P_3(t) = 3.677 + 0.5495e^{(-7.690t)}$	0.4260	0.0182
Cubic polynomial	$P_4(t) = -0.04504t^3 + 0.39912t^2 - 0.76433t + 3.62480$	0.2510	0.6591
Polynomial, degree 6	seven coefficients (Figure 14)	0.1103	0.9342
Damped sine	$P_5(t) = 4.01 - 5.6035e^{(-1.3198\tau)}\sin(0.8339\tau)$	0.0734	0.9709
Log-normal pulse	$P_6(t) = 4.01 - 1.38e^{(-(\ln(\tau/0.552))^2 \div 2(0.791)^2)}$	0.0590	0.9812

The exponential model fitted over the full record has the largest error, and an R^2 of 0.018 shows it explains almost none of the variation. The linear and cubic models are better but still poor, because neither can represent a latency period. The degree-six polynomial and the damped sine model both fit closely, but for different unsatisfactory reasons: the polynomial uses seven coefficients with no physical meaning, and the damped sine uses an amplitude larger than the pupil itself.

The log-normal pulse model has the smallest error of all, and it achieves this while being the only model whose parameters were not fitted to the curve at all. Its three constants come from the published metrics directly. This combination of accuracy and interpretability is why it is selected as the final model.

4. Applying the Model

The final model is

$$P(t) = 4.01 - 1.38 \cdot \exp[\, -(\ln((t - 0.2085) \div 0.552))^2 \div (2(0.791)^2)\,], \quad t > 0.2085$$

$$P(t) = 4.01, \quad 0 \leq t \leq 0.2085$$

In this model:

4.01 = initial pupil diameter in millimetres

1.38 = maximum amount of constriction

0.2085 = latency period before the main response begins

0.552 = time after latency at which maximum constriction occurs

0.791 = spread value controlling the width of the response

4.1 Checking the model at key points

At the moment of the light stimulus, $t = 0$, which is before the latency period ends, so $P(0) = 4.01$ mm. At the end of latency, $t = 0.2085$, the model gives $P(0.2085) = 4.01$ mm, showing the pupil is still close to its original diameter before the main constriction begins.

At the minimum point, t = 0.7605, first calculate
t − 0.2085 = 0.7605 − 0.2085 = 0.552
Substituting into the model,
P(0.7605) = 4.01 − 1.38 · exp[−(ln(0.552 ÷ 0.552))² ÷ (2(0.791)²)]
Since 0.552 ÷ 0.552 = 1 and ln(1) = 0, the exponential term becomes $e^0 = 1$, so
P(0.7605) = 4.01 − 1.38(1) = 2.63
This matches the published minimum pupil diameter. At the 75% recovery point, t = 2.2705, we have t − 0.2085 = 2.062, and substituting gives P(2.2705) ≈ 3.665 mm, matching the estimated recovery point derived from the published T75 value [4].

4.2 Predicted values

Table 4. Predicted pupil diameter values from the final log-normal pulse model

Time t (s)	Pupil diameter P(t) (mm)
0	4.010
0.2085	4.010
0.3000	3.906
0.5000	3.014
0.7605	2.630
1.0000	2.766
1.5000	3.235
2.0000	3.554
2.2705	3.666
2.5000	3.737

The table shows the overall pattern. The pupil remains at approximately 4.01 mm during latency, constricts rapidly to a minimum of 2.63 mm at approximately 0.7605 s, and then recovers, reaching approximately 3.665 mm at 2.2705 s.

4.3 Why the minimum occurs where it does

The time of minimum diameter follows from the structure of the model. The constriction term is largest when its exponent equals zero, which happens when
ln((t − 0.2085) ÷ 0.552) = 0
This means (t − 0.2085) ÷ 0.552 = 1, so t − 0.2085 = 0.552, giving
t = 0.7605

This confirms algebraically, without any numerical search, that the model reaches its strongest constriction at approximately 0.7605 s after the light stimulus, exactly as required by the general result derived in Section 2.6.

5. Conclusion

In this paper, pupil diameter after sudden exposure to light was modelled using continuous functions. Instead of treating pupil size as one fixed value, the model showed that pupil diameter passes through several stages: latency, rapid constriction, a minimum diameter, and recovery.

The model was based on anchor points reconstructed from published pupillary light reflex measurements [4]. The pupil began at approximately 4.01 mm, reached a minimum of approximately 2.63 mm, and recovered to approximately 3.665 mm at the estimated 75% recovery point. These values were not taken from a raw time series but reconstructed from published summary measurements including maximum and minimum diameter, latency, constriction velocity and T75 recovery time.

Several models were compared. The linear model was too simple, assuming a constant rate of change. The exponential model represented the constriction phase reasonably but could not show recovery, since a monotonic function has no turning point. The polynomial models could bend to fit the data, but their coefficients had no biological meaning and higher degrees produced overfitting. The damped sine model fitted closely but could suggest repeated oscillations, and required an amplitude larger than the pupil itself.

The final model chosen was the log-normal pulse model:

$$P(t) = 4.01 - 1.38 \cdot \exp[\, -(\ln((t - 0.2085) \div 0.552))^2 \div (2(0.791)^2) \,]$$

used for $t > 0.2085$, with $P(t) = 4.01$ for $0 \leq t \leq 0.2085$. It was selected because it gave the strongest combination of mathematical accuracy and biological meaning. Mathematically, it creates one main constriction response that reaches a maximum effect and then fades; the derivation in Section 2.6 shows this single turning point is guaranteed for every choice of parameters. Biologically, this matches the pupillary light reflex, which waits briefly, constricts rapidly, reaches a minimum and then recovers. The parameters have clear meanings: 4.01 is the starting diameter, 1.38 the maximum constriction, 0.2085 the latency, 0.552 the time to maximum constriction, and 0.791 the spread controlling recovery.

There are limitations. The values were reconstructed from published summary measurements rather than a complete raw recording, so the fitted errors measure agreement with a reconstruction and not with a direct observation. The model is based on group averages from adolescent participants, not on any individual, and pupil response is affected by age, tiredness, emotion, medication, light intensity, blinking and neurological condition. The model should not be used to diagnose any eye or brain condition.

Overall, this investigation shows that mathematics can describe a biological response over time. The pupillary light reflex is not a sudden jump from one pupil size to another but a continuous process with a measurable shape. By comparing parent functions on both fit and meaning, the log-normal pulse model was selected as the strongest representation of the reconstructed pupil-diameter data.

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Spatial Association of *Deschampsia Cespitosa* and *Ranunculus Repens* in Mercer Slough
By Ishan Perera

Research Question

What is the association between the distributions of tufted hairgrass (*Deschampsia cespitosa*) and creeping buttercup (*Ranunculus repens*) in Mercer Slough, as determined using quadrat sampling and a chi-square test?

Introduction

An ecosystem is composed of abiotic factors, such as weather, terrain, temperature, as well as biotic factors, the various organisms that live within the ecosystem. Whether an organism can survive in an ecosystem is based on the previously stated abiotic factors, since those determine whether a plant gets enough sunlight and water, or if an animal can maintain homeostasis, for example. The set of requirements of abiotic factors that a species needs to survive is called its fundamental niche (Matthiopoulos). However, these abiotic factors are not the only thing that shape where and how a species can survive. This is because an ecosystem is also composed of a large number of different species who all interact with each other. Whether it is predation, competition, or cooperation, the presence of other species changes the extent to which a species can survive (Pulliam). The role of a species in the ecosystem, such as how it interacts with other species, its habitat, and its behaviors, is called its realized niche.

In this essay, the realized niches of two specific plant species, *D. cespitosa*, and *R. repens*, will be studied, specifically how the two species interact with each other, and whether or not their growth is correlated with each other. As previously stated, there are three main types of interactions between species, predation, competition, and cooperation. Since both *D. cespitosa* and *R. repens* are on the same trophic level, neither of them predates the other, so predation will not be explored. Competition between species is a result of an overlap in fundamental niches. This means that both species strive to obtain the same resources from a limited resource pool, in the case of plants those resources are water, sunlight, and soil nutrients. This overlap in fundamental niches is one of the main shaping factors of the realized niche (Montana State University). The realized niche changes due to the overlap because each species adapts to avoid the limitations that the other species imposes, leading to each species growing in separate areas, or adapting to use other resources. While competition restricts the niche of a species, cooperation, or mutualism, does the opposite. By expanding the resource pool or provided resources, mutualism allows a species to grow its realized niche and survive in places/ways it would not normally (Rodrigues). This essay seeks to find which one of these the relationship between *D. cespitosa* and *R. repens* fits by examining their presence in the same habitat and searching them for positive or negative spatial association, which would suggest cooperation and competition, respectively.

D. cespitosa is a type of grass from the family Poaceae that is most commonly found from sea level to 14,000 ft in the northern hemisphere, though it is more present at higher

elevations. It prefers to grow in tidal marshes or on river bars, with a very high presence in wet meadows (Hill). It thus requires a primarily moist habitat, especially close to flowing water (FEIS). *R. repens* is a flowering plant from the family Ranunculaceae originally native to Europe and Northern Africa (New Zealand Plant Conservation Network). Thus, it is an invasive species in the area that this study takes place. It has a very large fundamental niche, being able to grow in many different habitats, provided it is damp (BSBI). Although the fundamental niche of *D. cespitosa* is much more restrictive, there is a clear overlap between the niches of the two species. Therefore, though there are some areas where *R. repens* would be able to thrive but *D. cespitosa* would not, in the areas where there is overlap, the two species would have some form of interaction. In the area being investigated, the Mercer Slough, the majority of it is an area that fits the fundamental niches of both species, meaning that on their own, they would both be able to thrive in a large portion of the area studied. By doing this, the possibility of the two species being separated simply because of different habitats is greatly decreased.



Figure 1. Tufted hairgrass (*Deschampsia cespitosa*) and creeping buttercup (*Ranunculus repens*).

Variables

Independent Variable

Presence of tufted hairgrass (*D. cespitosa*)

- This plant was chosen as the independent variable, since it was not a plant that was pervasive and had thus a much clearer niche compared to many other plants in the slough.

Dependent Variable

Presence of creeping buttercups (*R. repens*)

- Creeping buttercups were chosen since they grow in many different habitats, which allows for a more accurate judgment of spatial association between species as opposed to two species with distinct habitats.

Controlled Variables

Habitat

- Had to be controlled since the mercer slough is an area with a large amount of habitats, and if only one is sampled then it creates sampling bias and decreases the reliability of the conclusion
- Was controlled by widening the area data was collected from in order to ensure that multiple habitats were covered

Distance from water

- Had to be controlled because different concentrations of water in soil create different niches for plants, meaning that if only one is sampled, the data will be skewed towards certain plants, making any conjectures developed based on said data to be unreliable.
- This was controlled by taking data starting from next to the river, which was the main water source, and traveling away, sampling from all different distances from the river.

Distance from the path (human interaction)

- Since the mercer slough is a public park, it has a path that is commonly walked by humans. This means that the areas next to the path were more likely to have been affected by humans at some point, whether that is from litter, spilt water/food giving resources, or plants being killed due to being stepped on.
- This was a variable that could not be controlled, since straying from the path is banned by law. The closest to controlling the variable was putting the quadrat as far from the path as possible without actually leaving the path.

Preliminary experiments

When going to collect preliminary data, a different type of grass was initially selected for the experiment. However, initial data suggested that since it was incredibly common and shared a similar niche to *R. repens*, there was no spatial association between the two species, positive or negative. This meant that for the actual experiment, the species had to be changed, with *D. cespitosa* being chosen, since a small sample of data had shown it was most likely to have a spatial association with *R. repens*. Additionally, in the area sampled, both species had a relatively high frequency, which was what led for it to be chosen.

Hypothesis

Based on the initial observations from the preliminary experiments, the hypothesis was that there would be a negative spatial association between *D. cespitosa* and *R. repens*. While this hypothesis was formulated based on trends in the preliminary data, it was also due to the fact that while the *D. cespitosa* and *R. repens* have overlapping fundamental niches, they appear to have different enough realized niches that the areas that they grow in have limited overlap.

Methods

The method used to determine spatial association between the two species in this experiment was quadrat sampling and a chi square test. A chi square test is a mathematical process that compares a model to actual observed results. In this experiment, the chi square test

can be used to compare the expected data based on the null hypothesis (no spatial association between the species), to the actual data that is collected. Since the chi square test will be used, the data that shows the presence of a species must therefore be quantified. The easiest way for this to be done is to use quadrat sampling. Quadrat sampling in this experiment involves using a square quadrat in order to see if a species is present in a small area. By repeating this and recording how many quadrats one or both of the species are present in, quantitative data is collected.

Equipment needed

- 0.5m by 0.5m quadrat
    - Quadrats should be made from solid material like PVC pipes or metal. It is also possible to use tape or something similar, but when placing the quadrant, one must ensure that it is fully extended so as to not accidentally measure a smaller area.
- Ruler or measuring tape capable of measuring 1 or more meters

Procedure

Choosing a location

1) Choose the larger area that will be observed (such as the Mercer Slough)
2) Within that area, find the two species that will be studied for spatial association
3) To control distance from the water along with differences in habitat, select a route through the area that covers multiple habitats that are not too similar, i.e. all along a river.

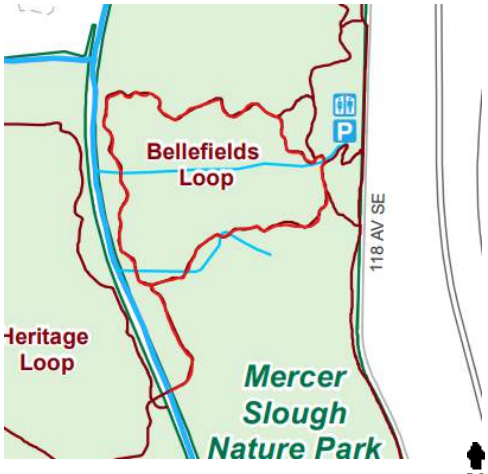


Figure 2. Sampling route through Mercer Slough.

In Figure 2, it is shown that the distance from the larger river as well as that from the creeks is varied. Though it cannot be seen in the figure, the habitats were varied as well, with the northeastern portion of the trail having a higher elevation and the northern area being much more shaded. Though varied, the habitats are not and should not be completely different, since the two species must still be present and able to be monitored.

Collecting data

1) Starting from an area close to the main source of water, place the quadrat off the side of the path, ensuring that it is fully laid out and laid flat on the ground
    - Follow the route away from the water source in order to make sure that the distance from a water source is a controlled variable
    - Alternatively, the route could go from the furthest point from the water source towards the water source.
2) Record whether or not the species are present within the quadrat
3) Using the ruler or measuring tape, move a precise distance along the path from the quadrat (5 meters was what was used in this experiment, but the distance should depend on the amount of distance available for data collection)
4) Place the quadrat at the end of said set distance and repeat steps 1-3
    - This should be repeated for the number of trials that are desired (more trials will lead to a more precise chi squared test, so 200 trials were done for this experiment)

Research methodology

Before this procedure was created, research had to be done to determine how spatial association should be measured as well as specific variables that were to be used. The larger outline for the procedure was found in the textbook Biology for the IB Diploma Third Edition by C. J. Cleggs and Andrew Davis (Clegg). This textbook provided the idea for using quadrat sampling as well as a chi squared test to test for spatial association between the species. The specifics for the procedure were determined based on the area that was to be sampled, Mercer Slough, which determined the size of the quadrat, the distance between quadrats, and the types of areas sampled.

Risk assessment

Hazard	Risk	Precautions taken
Flora within the Mercer Slough	Possible irritation to the skin when contacted directly	No direct contact with plants made even when placing/moving the quadrat, unless the species contacted is known to be safe
Fauna within Mercer Slough	Certain species become aggressive if threatened. Certain insects present such as mosquitoes can carry diseases.	All safety guidelines are to be followed in regards to local animals, this means pausing data collection if a possibly dangerous animal is spotted. Insect repellent is used to prevent mosquito bites.
Sun	Risk of sunburn or dehydration in areas with lots of sun or high temperatures	Sunscreen applied to all exposed skin, dressed appropriately for the weather.
Natural water	Pollution or contaminants could be dangerous if ingested. Larger rivers can be dangerous if fallen into.	Water was not consumed. Make sure to follow the path carefully and not do anything that could lead to falling into the river.

Ethical and environmental concerns

In a field study such as this experiment, it is crucial to take steps to ensure that the study does not have any lasting impact on the organisms and ecosystems being studied. In this experiment, the main concerns would be the legal implications of working in a government-protected park, along with those of working with plants. The only legal requirement that had to be followed in this experiment was that one had to stay on the marked path, which was what made limiting human impact on the studied species difficult. The other ethical concern is that which comes with studying live plants. The steps taken to ensure the safety and lack of permanent impact on the change were the choice of quadrat material and quadrat placement. In this experiment, the quadrat was made from blue masking tape, which provided a clear quadrat that did not squish or bother the plants when placed. Even though this quadrat could be placed directly on plants without harming them, the quadrat was still carefully placed so as to directly touch as few plants as possible.

Results

Raw data

Table 1. Observed frequency of *D. cespitosa* and *R. repens* across 200 quadrats.

	# of quadrats with *R. repens*	# of quadrats without *R. repens*	total
# of quadrats with *D. cespitosa*	1	8	9
# of quadrats without *D. cespitosa*	96	95	191
total	97	103	200

Processed Data

To calculate the expected amount of each species if there was no spatial association and the species were randomly distributed with respect to each other, the following equations are used:

$$P(Species\ A) = \frac{\#\ of\ quadrats\ with\ species\ A}{\#\ of\ quadrats}$$

$$P_{expected}(Species\ A\ \&\ Species\ B) = P(Species\ A) \times P(Species\ B)$$

To calculate the Chi Square value, the following equation is used:

$$\chi^2 = \frac{(Observed - Expected)^2}{Expected}$$

The summation is for each type of result, only species A, only species B, both species A and B, or neither species A nor B.

Sample calculations:

$$P(R.\ Repens) = \frac{97}{200} = 0.485$$

$$P_{expected}(R.\ Repens\ \&\ D.\ Cespitosa) = 0.485 \times 0.045 = 0.022$$

Table 2. Expected frequency of *D. cespitosa* and *R. repens* across 200 quadrats.

	Expected # of quadrats with *R. repens*	Expected # of quadrats without *R. repens*	Total
Expected # of quadrats with *D. cespitosa*	4.365	4.635	9
Expected # of quadrats without *D. cespitosa*	92.635	98.365	191
Total	97	103	200

$$\chi^2 = \frac{(1-4.365)^2}{4.365} + \frac{(8-4.635)^2}{4.635} + \frac{(96-92.635)^2}{92.635} + \frac{(95-98.365)^2}{98.365} = 5.27$$

Table 3. Degrees of freedom versus χ^2 critical values

df	$\chi^2_{.995}$	$\chi^2_{.990}$	$\chi^2_{.975}$	$\chi^2_{.950}$	$\chi^2_{.900}$	$\chi^2_{.100}$	$\chi^2_{.050}$
1	0.000	0.000	0.001	0.004	0.016	2.706	3.841
2	0.010	0.020	0.051	0.103	0.211	4.605	5.991
3	0.072	0.115	0.216	0.352	0.584	6.251	7.815
4	0.207	0.297	0.484	0.711	1.064	7.779	9.488
5	0.412	0.554	0.831	1.145	1.610	9.236	11.070

Watkins, J. (n.d.). Chi-square table. University of Arizona. https://math.arizona.edu/~jwatkins/chi-square-table.pdf

$$Degrees\ of\ Freedom\ =\ (\#\ of\ rows\ -\ 1)\ \times\ (\#\ of\ columns\ -\ 1)\ =\ 1$$

The chi square value is used to determine whether the null hypothesis that the species are randomly distributed is true. Since the calculated chi square value of 5.27 is greater than that of the $\chi^2_{.050}$ of 3.841, this means that there is a less than 5% chance that the calculated spatial association is based on random chance. This means that it can be concluded that the null hypothesis should be rejected, and there is a statistically significant spatial association between *D. cespitosa* and *R. repens*.

Discussion

In the processed data, it is proven that there is most likely a significant spatial association between *D. cespitosa* and *R. repens*. Additionally, in the raw data, it is shown that there was only one quadrat with both species present, while the presence of only one species was much more common, which is what the high chi-squared value proves. This implies that the significant spatial association between *D. cespitosa* and *R. repens* is a negative one. This means that the

hypothesis of the two species having a negative correlation is correct. However, in the raw data, there is an overall low number of *D. cespitosa*. This means that any error would have a proportionally larger impact on the results, creating some uncertainty about the strength of the spatial association.

The hypothesis stated that the two species most likely have overlapping fundamental niches, since they live in similar areas with similar abiotic factors, but that they also do not seem to have overlapping realized niches. Since this was confirmed by the data, it implies that the relationship between the two species is most likely to be that of competition. However, while competition is the most likely biological explanation, it is not the only one. The data shows that the two species' realized niches are different, since they have a negative spatial association, it is important to note that correlation does not equal causation, meaning that the interaction between two species is not necessarily what caused that difference.

Though the relationship implied by the differing realized niches is competition, these two plants are not the only species in the slough. This means that the realized niches of each species are not only shaped by interactions with each other, but also interactions with the other species in the habitat. Since both *D. cespitosa* and *R. repens* are on the first trophic level, they thus do not predate on other species, so other species being used as prey is not a factor that could shape their realized niches. However, a factor that could shape their niches is predation. The two species would have a negative spatial association if the species that they are each predated by have different realized niches. Since the species would each be much more present in areas where their predators are not, if their predators are each in opposite areas — if the predators themselves have a negative spatial association in their habitats — then the two species being investigated would have a negative spatial association. However, since there are a large number of constraints for this explanation to be true for the data collected, it is highly unlikely that this is the best explanation for the reason for negative spatial association between the two plants.

The last explanation is that the two species have differing fundamental niches, inherently live in slightly different areas, therefore having a negative spatial association. A reason for this is that the area investigated could have crossed the boundary between their habitats, with the slight number of one of the species in the other habitat being caused by edge effects. In this study, that would be prevalent since *D. cespitosa* prefers water, meaning that it would be the most prevalent in the areas near the water. On the other hand, *R. repens* does not share this tendency, and would thus have a habitat further from the water. At the edge of their habitats, edge effects would mean that there would be some overlap in the habitats, which is why there were still some quadrats where both were spotted.

Another possible explanation for differing fundamental niches is that although most of the abiotic factors are the same, there could be factors that were not accounted for in the data that differ between the fundamental niches of each species. Additionally, as shown in the background, the majority of the slough and almost all of the area investigated fit the fundamental niche of both species. Thus, this explanation would only work if there was some form of an external factor that was not explored, such as acidity of the soil, that changes throughout the

slough. However, given the slough's status as a protected nature reserve, it is highly unlikely that any of those factors would be an extreme that would prevent any one of the species from surviving. This is especially true since for this explanation to be true, that factor would have to vary by a significant amount throughout the slough, since both species were clearly present within the slough.

Conclusion

Out of these possible explanations, the ones that are most likely are that of competition or that of the two having separate habitats that were both in the area studied. Looking at how the terrain varies throughout the path investigated, the second explanation is more likely. Thus, we conclude that the relationship between *R. repens* and *D. cespitosa* is that they are both species that live in different habitats within the same larger ecosystem.Though it must be noted that the chi-squared test only proved spatial association and not the cause for that association.

Evaluation

The main types of error involved in this study were systematic error, random error, and human error.

The main cause of systematic error in this study was the fact that the quadrats could not be far from the path and were therefore measuring the plants that had the most amount of human interference. This meant that the spatial association being measured might not exist in an ecosystem without said interference. For example, if one species is more susceptible to pollution than the other, then it would appear that there is some form of interaction between the two species, when in reality it was an interaction between the species and the presence or lack of pollution.

Another source of error is random error that comes with random sampling. This means that since the quadrats were placed randomly, there is always a small chance that the measured spatial association was gained purely off coincidence. Thus, had the quadrats been placed differently, or more quadrats were used, then a different conclusion would be drawn. Since 200 quadrats were used, it is highly unlikely that random error had a large effect on the data, however, the relatively low number of *D. cespitosa* means that it is still possible that random error influenced the final calculation.

The last type of error in this experiment is human error. The main case of this would be when examining the quadrats, a species that was present was not seen and thus changed the results of the experiment. The other case, which is even less likely, is a species not being present but being marked as present. This would happen if a very similar looking species was mistaken for one of the two being sampled. In this experiment, an extra sighting is more likely to happen with *D. cespitosa*, since it is of a family that is quite common in the slough, with many of the plants looking very similar (especially when not bloomed). Although steps were taken to avoid this—taking pictures every couple of quadrats to re-analyze later, as well as obviously taking much care when observing a quadrat at any given time—it is still possible that over the course of

200 quadrats that the presence of a species was missed in a couple of them. Although this would normally have a very minor effect on the results, due to the large number of quadrats, the scarcity of *D. cespitosa* means that even one extra or one less sighting of the species would have a very large effect on the chi-squared value. This means that on the off-chance that this error was present, the real chi-squared value could either be much higher, signaling even stronger spatial association, or much lower, giving a false correlation. The extent of the effect of the error would also depend significantly on whether or not both species were present, since that determines whether it changes the results or not.

If this study were to be done again, there would need to be some procedural improvements in order to reduce all of these causes of uncertainty. In this study, the error only created high amounts of uncertainty due to the relatively low number of *D. cespitosa*. Therefore, to reduce this, either a more prominent species should be chosen, more quadrats should be taken in a larger area, or a different location should be used in order to reduce the uncertainty that comes with a low presence of one species. However, 200 quadrats is already a large enough sample size for most species, as seen with *R. repens*, and any more would be extremely time-consuming, especially with the limited area that can be explored in the slough, therefore the most efficient improvement while still researching the same question would be to find an area with a higher presence of both species. The other main source of error came from the legal problems that arise when researching within a nature reserve. This is an issue that cannot be easily solved with resources accessible to a student. However, in an optimal situation, an area with very limited human interaction could be found where the study could be conducted legally, thus removing the error that comes from an area that is commonly interacted with by humans.

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Capsule Endoscopy as a Supplementary Screening Tool for Colorectal Cancer
By Richard Tsao

Abstract

Colorectal cancer (CRC) is one of the most prevalent and preventable cancers in the United States, with approximately 150,000 new diagnoses and over 50,000 deaths annually. Despite the proven efficacy of traditional colonoscopy, the number of patients getting screened remains low due to discomfort, social stigma, and inconvenience. Capsule endoscopy has emerged as a promising non-invasive supplementary tool; however, existing commercial devices have multiple limitations. These limitations include polyp detection accuracy, image resolution, and the convenience of data retrieval. This paper reviews two leading capsule endoscopy systems, CapsoCam SV-1 and the PillCam Colon 2, analyzes their technical capabilities and limitations, and proposes an improved capsule device design. The proposed design utilizes a higher-resolution camera (OmniVision OV9734), enhanced LED illumination, Bluetooth-enabled wireless data transmission, and time-of-flight depth mapping via infrared projection. This proposed device is not intended to replace colonoscopies but to serve as an accessible, at-home screening tool that encourages patients to seek further clinical evaluation if necessary.

Keywords

capsule endoscopy, colorectal cancer, gastrointestinal mucosa, optical imaging, Time-of-Flight imaging, polyps, device configurations, prototype design, cancer screening, device performance, social stigma.

1. Introduction

According to the National Cancer Institute and the Centers for Disease Control and Prevention, approximately 150,000 individuals in the United States receive a colorectal cancer diagnosis each year, and more than 50,000 die from the disease annually. Yet, these numbers are largely preventable through routine screenings (National Cancer Institute; Centers for Disease Control and Prevention).

A particularly concerning trend is the rise of colorectal cancer among individuals under the age of 50. Dr. Patel S. G. analyzed Surveillance, Epidemiology, and End Results (SEER) Program data from 1980 to 2016 and documented a sustained increase in CRC incidence among adults under 50, with rectal cancer rates rising especially sharply in those aged 30 to 39 (Shah and Patel). More recently, a review published in EBioMedicine reported that while CRC incidence among older adults declined by approximately 1% per year between 2012 and 2021, rates increased by 2.4% per year among those under 50 during the same period. This trend was observed across more than 50 countries, demonstrating the persistent issue around colon cancer (Loomans-Kropp and Umar).

These trends prompted the American Cancer Society to lower its recommended screening initiation age from 50 to 45 years in 2021 (Johns Hopkins Bloomberg School of Public Health).

However, even with the updated recommendations, convincing patients to get a colonoscopy remains a significant public health challenge. One reason for this may be the perceived social stigma against colonoscopies. Dr. Wenjie Zhao describes "individuals internalizing socially negative labels into self-perception, avoiding screening due to the anticipation of discrimination, and implicit discrimination embedded in social systems" (Zhao et al.). This in turn results in a lower number of patients coming in for screenings.

Another contributor to the lack of individuals receiving screening is related to the diagnostic technologies. Traditional colonoscopy is widely regarded as the gold standard for colorectal cancer detection due to its high diagnostic accuracy and capacity to simultaneously detect and remove polyps (Bharwad et al.). This procedure requires sedation, bowel preparation with a large-volume cleansing solution, and patients must plan their day around extensive bowel movements. As noted by Dr. John M. Inadomi, "colonoscopy's invasive nature and burdensome preparation are primary drivers of low patient compliance, ultimately delaying diagnosis" (Inadomi et al.).

Capsule endoscopy has been proposed as a less invasive add-on to traditional colonoscopy. In this procedure, the patient swallows a pill-sized camera that traverses through the gastrointestinal tract, capturing images without the need for sedation or instrumentation.

This paper examines two commercially available capsule systems—the CapsoCam SV-1 and the PillCam Colon 2. After analyzing their technical capabilities and clinical performance data, it concludes with a proposed design that addresses key deficiencies in the current generation of devices including low image quality, and 2d imaging.

2. Review of Current Colonoscopic Devices

2.1 The CapsoCam SV-1

The CapsoCam SV-1 (CapsoVision, Inc.) is an ingestible capsule endoscopy system cleared by the FDA for visualization of the small-bowel mucosa in adults, and not specifically indicated for colonic polyp screening (U.S. Food and Drug Administration, "CapsoCam"). This is important when understanding its performance data in the context of colorectal cancer screening. The device, according to the FDA, measures 11.3 mm in diameter and 30.5 mm in length and is equipped with four laterally positioned cameras that together provide a 360-degree panoramic view of the gastrointestinal mucosa as the capsule traverses the tract (U.S. Food and Drug Administration, "CapsoCam"). Upon ingestion, the device records and stores images in onboard flash memory. Battery life, according to the FDA, supports approximately 15 hours of operation, and storage capacity accommodates approximately 32,000 to 50,000 images at a resolution of 128 × 896 pixels. Frame rate begins at 5 frames per second (FPS) per camera for the first two hours and decreases to 3 FPS per camera thereafter. A notable feature of the CapsoCam SV-1 is that it can be self-administered at home, affording patients a degree of privacy.

The device has several significant limitations. First, data retrieval requires manual recovery of the capsule from fecal matter, a process that is unpleasant, time-consuming, and burdensome. Second, reviewing the tens of thousands of captured images demands considerable physician time, increasing the procedure's cost. The cost of the CapsoCam SV-1 capsule ranges from $800 to $1,000, and physician interpretation fees range from $115 to $825, yielding a cost of approximately $1,020 to $3,000 (U.S. Food and Drug Administration, "CapsoCam").

Clinical performance data from the FDA 510(k) submission demonstrate the device's accuracy compared with the PillCam SB2 in subjects with suspected small bowel disease. Overall, "Positive Agreement was 69.05% (95% CI: 53.97 -- 80.93%) and Negative Agreement was 81.43% (95% CI: 70.77 -- 88.81%)" (U.S. Food and Drug Administration, "CapsoCam"). For specific diagnostic categories within the small bowel, Vascular Positive Agreement was "66.67%, Ulcerative Positive Agreement was 64.29%, and Mass/Polyp Positive Agreement was 0.00% (95% CI: 0.00–48.99%)" (U.S. Food and Drug Administration, "CapsoCam"). It is important to note that this 0% figure reflects the detection of small bowel masses and polyps, which further highlights the need for improvement.

2.2 The PillCam Colon 2

The PillCam Colon 2 is a two-component system consisting of an ingestible capsule and an external data recorder worn as a vest. Unlike the CapsoCam SV-1, the PillCam Colon 2 was specifically designed and cleared for colonic visualization. According to Iddan and Sturlesi, and Wang and Wilson, the capsule measures 31.5 mm in length and 11.6 mm in diameter and is equipped with two cameras—one at each end—each providing a 170-degree field of view (Iddan and Sturlesi; Wang and Wilson). Battery life supports up to 10 hours of recording, sufficient for colonic transit in most patients. The device employs an adaptive frame rate, capturing images at 4 FPS during slower transit and increasing to 35 FPS during faster movement. Unlike the CapsoCam SV-1, the PillCam Colon 2 capsule is disposable and does not require retrieval after use; image data is stored in the external vest recorder and transferred to a physician for review.

The main limitation of the PillCam Colon 2 is its inability to provide a full 360-degree view of the colonic mucosa, as the two end-facing cameras create blind spots along the lateral walls. This may result in the missed detection of polyps, even in regions not obscured by mucosal folds. Physician review of the full image set remains required, contributing to higher procedural costs, and the capsule's disposable nature increases per-unit manufacturing expense.

Clinical performance data indicate that the PillCam Colon "2 correctly identified polyps 6 mm or greater in 68.8% of cases (sensitivity) with a specificity of 81.3%, and polyps 10 mm or greater in 64.9% of cases with a specificity of 92.9%" (U.S. Food and Drug Administration, "De Novo"). A 2025 systematic review and meta-analysis of 12 prospective studies comprising 4,316 patients found that second-generation capsule endoscopy demonstrated pooled sensitivity and specificity of 0.89 and 0.94, respectively, for polyps 6 mm or greater, and 0.91 and 0.98 for polyps 10 mm or greater, suggesting that newer-generation devices perform considerably better than earlier models. A meta-analysis of multicenter studies similarly concluded that

second-generation colon capsule endoscopy demonstrated high sensitivity and specificity relative to colonoscopy, while acknowledging that the comparatively higher cost and bowel preparation requirements remain barriers to widespread adoption (Câmara et al.).

3. Proposed Design Components and Analysis

Building upon the limitations of existing capsule endoscopy devices, a new capsule design is proposed that incorporates updated components to enhance image quality, reduce patient discomfort, and improve three-dimensional visualization of the colon (Figure 1).

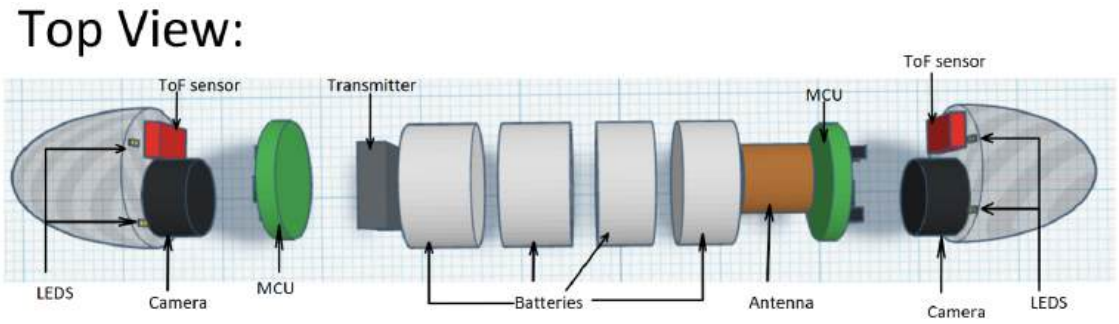


Fig 1: A top-view model of the proposed capsule endoscope showing the internal places of all components along the axis. Rendering generated using Tinkercad. ToF = Time of Flight; MCU =Microcontroller Unit.

3.1 Capsule Dimensions

The proposed device requires approximately 151.32 mW for up to 8 hours of operation (Table 1), accounting for two cameras, 2 Time of Flight (ToF) ranging sensors, eight LEDs, one antenna, and one transmitter (Figures 3a-3f). The capsule diameter was increased from an initial target of 9.9 mm to 11.9 mm (Figure 2), and the capsule length was extended to 36.6 mm (Figure 1). Although these dimensions exceed standard capsule size guidelines of 9.9 mm x 26.1 mm (“Capsule Size Chart”), regulatory precedent exists for larger capsule endoscopes. The FDA-cleared PillCam Colon 2 measures 12 mm x 33 mm (“FDA Clears Given Imaging’s PillCam Colon 2”), and the proposed device—at 11.9 mm x 36.6 mm—has a smaller diameter than the PillCam Colon 2 and only slightly greater length. The dimensions of this device are similar to the proposed device, making approval plausible.

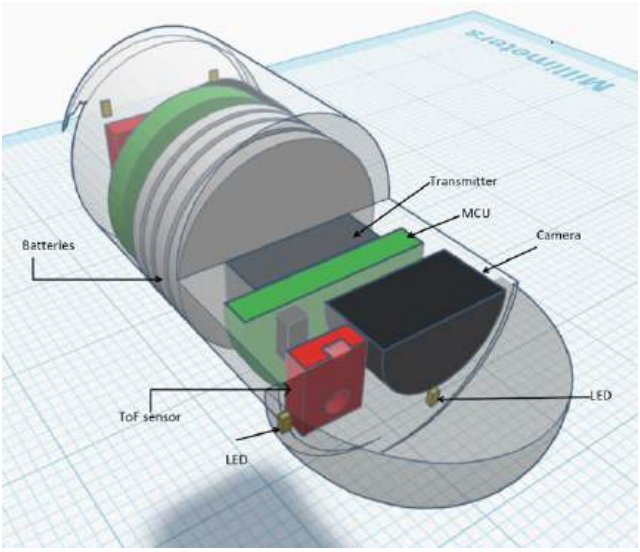


Fig 2: A 3-dimensional cutaway of the proposed capsule endoscope which shows the internal components and arrangement within the capsule. Rendering created using Tinkercad.

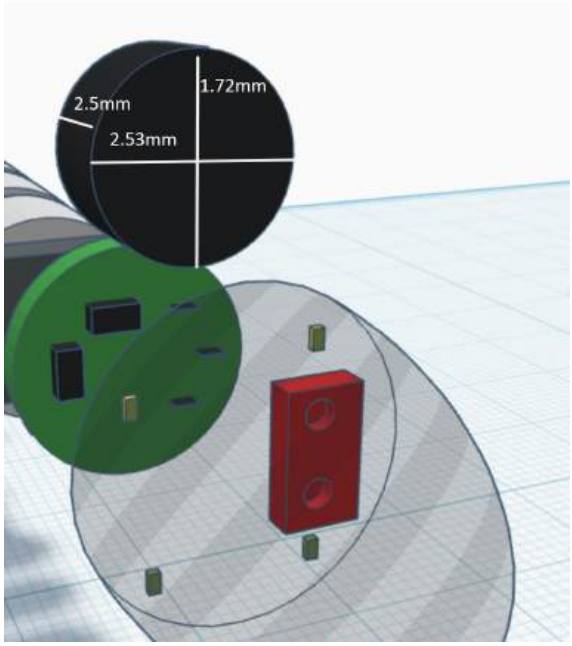


Fig 3a: A CAD model of the Omnivision OV9743 camera and encoder module (2.53 x 1.72 x 2.5 mm) shown in the front near the ToF sensors and LEDs. Rendering generated using Tinkercad.

3.2 Camera Module

This proposed device uses the OmniVision OV9734 image sensor, which captures images at 720p high-definition video at 30 FPS (OmniVision Technologies). This represents a substantial improvement over the image resolutions of both the CapsoCam SV-1 and the PillCam Colon 2. The OV9734 integrates the processor and sensor within a single 2.53 x 1.72 x 2.5 mm

module (Figure 3a), making it well-suited for the spatial constraints of a capsule. Power consumption remains below 69 mW during recording.

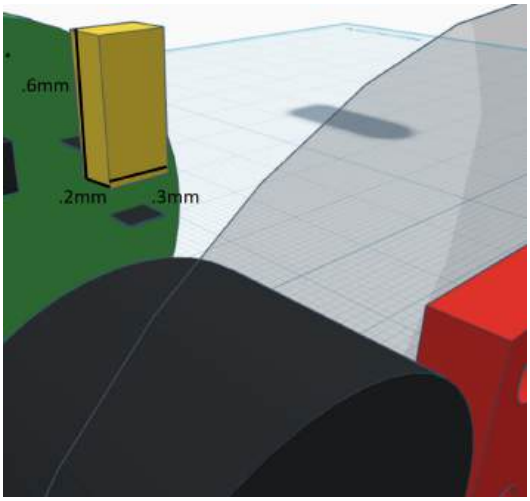


Fig 3b: CAD model of the LITEO N 859-LTW-FC03DCD5 LED units (0.6 x 0.3 x 0.2 mm each) shown next to the Tof sensors and camera module. Rendering generated using Tinkercad

3.3 Illumination

This proposed device uses high-intensity LEDs with a luminous intensity of 710 mcd and compact dimensions of 0.6 x 0.3 x 0.2 mm, consuming 62 mW per unit as seen in Figure 3b (LITEON Technology Corporation). The small form factor allows for the inclusion of multiple LED units to ensure a sufficient amount of light throughout colonic transit.

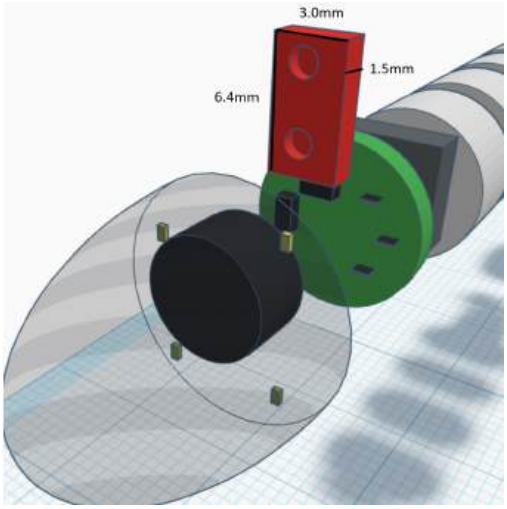


Fig 3c: CAD model of the VL53LOX ToF sensor (6.4 x 3.0 x 1.5 mm) shown adjacent to the camera module. Rendering generated using Tinkercad

3.4 Three-Dimensional Imaging

Most commercially available capsule endoscopes produce only two-dimensional images, which can limit a physician's ability to assess the imaging provided. Penne et al. described the

first application of the time-of-flight (ToF) measurement principle via endoscope optics, laying the groundwork for a new generation of three-dimensional endoscopes. In the ToF method, an infrared light source illuminates the scene with intensity-modulated light; each pixel of the ToF sensor measures the phase delay between the emitted and reflected signals, which is proportional to the distance traveled by the light. From this phase delay, the time is calculated, and using the known speed of light, the distance from the endoscope tip to the tissue surface is derived for every pixel simultaneously. This produces a real-time 3D image without requiring any modification to the endoscope optic itself (Penne et al.). In in vitro experiments conducted in a porcine stomach, Penne et al. achieved a mean depth measurement precision of 0.89 mm at 20 frames per second with 3,072 three-dimensional points, and successfully reconstructed the dimensions of the structures, demonstrating the method's potential for measuring tumors and other features. A 2024 systematic review of real-time three-dimensional reconstruction methods for medical endoscopy further confirmed that ToF sensors can be used (Richter et al.). Stereo depth imaging was considered but rejected, as it requires two spatially separated cameras, a configuration not feasible within the capsule's dimensional constraints.

This proposed device incorporates ToF imaging using the VL53L8CX ToF sensor (Figure 3c). In the ToF configuration (Figure 2), the infrared projectors emit modulated light that reflects off the colon, and the sensor then calculates the distance to each point. It will then follow a similar process described above, providing an 8 x 8 grid of 64 distance measurements over a 63° field of view at up to 15 FPS. This produces a 64-point depth contour map (STMicroelectronics). While this does not produce a full 3d model demonstrated by Penne et al., it provides sufficient depth information to estimate polyp height, size, and shape. This has the potential to reduce diagnostic errors associated with two-dimensional images. The information is critical for clinical decision-making regarding whether a patient should be referred for a full colonoscopy.

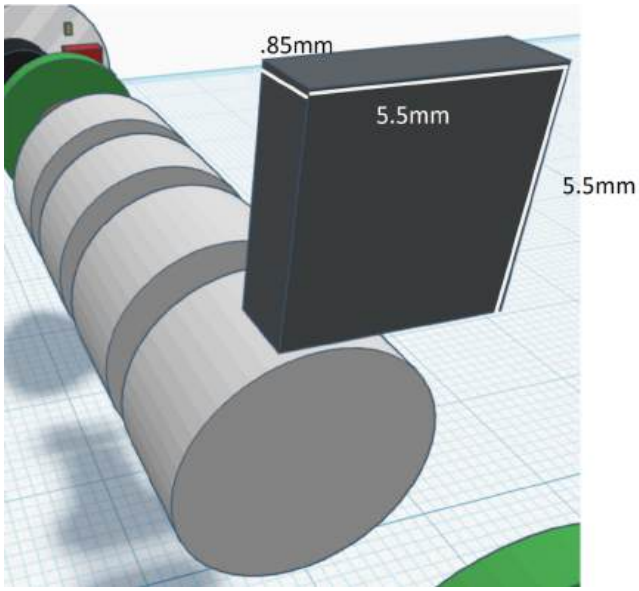


Fig 3d: CAD model of the Nordic nRF52832 Bluetooth Low Energy transmitter (5.5 × 5.5 × 0.85 mm) shown near the battery stack and close to the processor. Rendering generated using Tinkercad

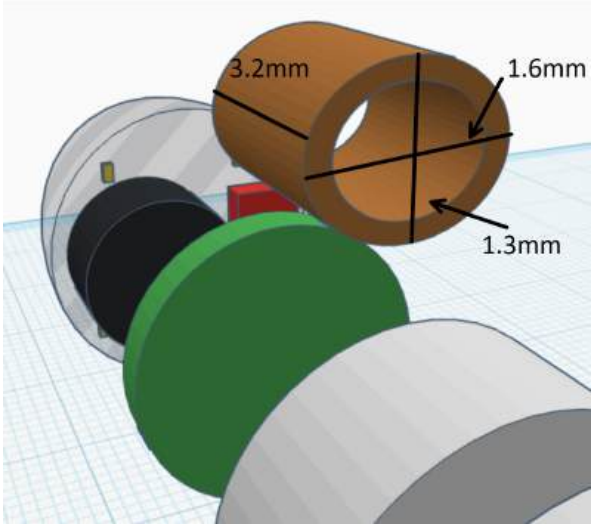


Fig 3e: CAD model of the Johanson 2450AT18A100 chip antenna (3.2 × 1.6 × 1.3 mm) shown in the mid-section of the capsule adjacent to the battery stack. Rendering generated using Tinkercad

3.5 Wireless Data Transfer

The proposed device eliminates the need for physical device recovery and manual data transfer. The proposed capsule incorporates a Bluetooth-enabled antenna operating in the 2.4 GHz band with a gain range of 0.5 to 1.5 dBi and a power requirement of 2.5 to 100 mW (Johanson Technology). This antenna is paired with a Nordic Semiconductor Bluetooth Low Energy chip that contains a microcontroller plus BLE radio (Nordic Semiconductor), enabling real-time image transmission to a paired mobile device (Figures 3d-3e). The mobile device would need to be in close proximity to the body due to transmission loss from inside the human body. This allows physicians to receive and review images without physically retrieving the capsule. This will significantly improve the patient's experience and procedural efficiency.

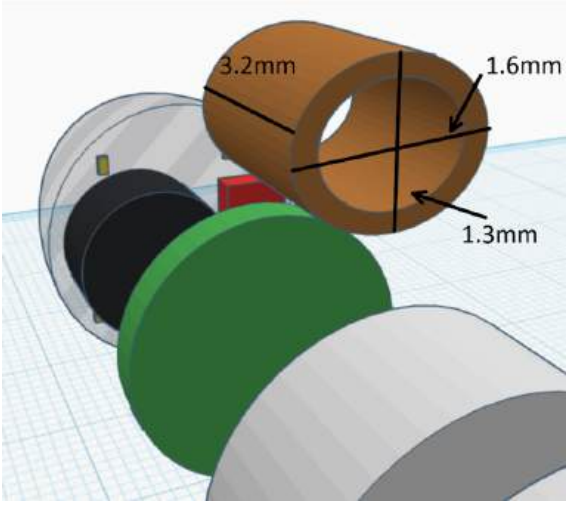


Fig 3f: CAD model of the four stacked SR44 silver oxide batteries (11.6mm diameter X 5.4mm per cell, 21.6mm total stack height) occupying the center of the capsule. Rendering generated using Tinkercad

3.6 Battery Configuration

This device utilizes the four silver oxide SR44 Batteries (figure 3f; 200 mAh each with 1.55V; 11.6 mm diameter × 5.4 mm height), providing a total capacity of 1240 mAh (Energizer). This amount of power isn't enough to run the capsule for the whole duration while ingested. However, this can be addressed through duty cycling–activating power-intensive components as seen in table 1. Note the existing capsule endoscopic devices also use duty cycling.

Table 1

Duty Cycle & Power

Subsystem	Part	Active Power	Avg Power assuming 13% Duty cycle
Image sensor x2	Omnivision OV9734	138 mW	17.94 mW
ToF sensor x2	VL53L8CX	430 mW	55.9 mW
White LEDs x8	LITEON LTW-FC03DCD5	496 mW	64.48 mW
BLE & MCU	nRF52832	100 mW	13 mW
Total		1164 mW	151.32 mW

Table 1 conveys the power of the devices and the duty cycle percentage needed to achieve an 8-hour battery life.

4. Intended Clinical Role

The proposed device is not intended to replace traditional colonoscopy, which remains the gold standard for colorectal cancer detection because of its ability to simultaneously detect and remove polyps (Bharwad et al.). This device is conceived as an accessible, at-home screening tool. As illustrated in Figure 4, patients would self-administer the capsule privately, with image data transmitted wirelessly to a paired mobile device and subsequently forwarded to a physician for remote analysis. Should the physician identify suspicious findings, the patient would then be referred for a traditional colonoscopy or surgical evaluation.

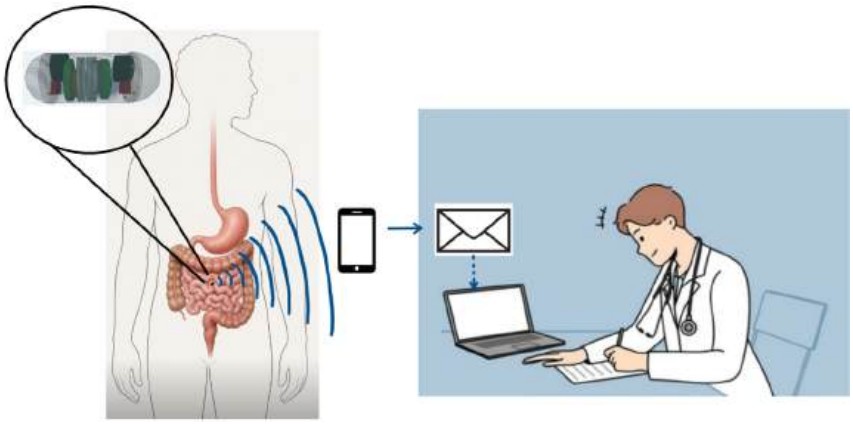


Fig 4: A schematic representation of the proposed device workflow. The capsule transmits imaging data to the phone—wirelessly via Bluetooth—which then forwards it to a physician for remote review.

This approach has the potential to increase the number of people who get colon screenings. It would particularly be effective among patients who would otherwise avoid colonoscopy entirely due to discomfort, embarrassment, or inconvenience. It is important to consider that the proposed device is improving upon previous capsule endoscopes.

5. Discussion

The increasing diagnosis of colorectal cancer among younger populations demonstrates the urgent need for screenings that are both effective and acceptable to a broader patient population. While colonoscopy remains irreplaceable, public noncompliance poses a challenge and underscores the need to develop supplementary tools.

Fully autonomous robotic endoscopy systems have been proposed as a long-term alternative; however, the engineering challenges involved in developing a cost-effective endorobot that meets clinical requirements remain substantial (Manfredi). These comparisons show that the traditional colonoscopy is favored more overall. Capsule endoscopy represents a more immediately feasible avenue. Meta-analytic evidence suggests that “second-generation colon capsule endoscopy is achieving sensitivity and specificity values approaching those of traditional colonoscopy for clinically significant polyps” (Câmara et al.).

The proposed design, therefore, was systematically developed to address the specific shortcomings identified in commercial devices. The most critical limitations of current devices are limited image resolution, absence of three-dimensional visualization, and cumbersome data retrieval. Wireless transmission eliminates the need for physical capsule recovery, improving both patient experience and procedure for nurses. ToF depth map imaging offers the potential for more accurate and higher quality partially 3D images. Table 2 summarizes the differences between the proposed device and the other devices examined in the paper.

Table 2

Comparison of Capsule Systems

Feature	CapscoCam SV-1	PillCam Colon 2	Proposed Device
Cameras	4 (lateral)	2 (ends)	2+ToF
Resolution	128 x 896 px	256 x 256 px	1280 x 720 px
Field of View	360°	344°	344°
3D Imaging	no	no	64-point ToF depth map
Data Transfer	Internal storage	External vest	Bluetooth

Battery Life	15 hours	10 hours	8 hours

There are limitations of the proposed design, which include the inability to insufflate the colon, which may allow polyps concealed within mucosal folds to remain undetected (a challenge shared by all passive capsule endoscopy devices). Additionally, the proposed capsule exceeds standard size guidelines, which presents a challenge in meeting regulatory approval. However, the Pillcam also doesn't meet the size guidelines, making this proposed device plausible nonetheless. Third, the proposed Bluetooth link is power-efficient but bandwidth-constrained. At 2.4 GHz in-body attenuation is significantly higher than sub-GHz radios. This is why the existing Pillcam uses a body-worn receiver. The images and videos must be compressed to fit within these constraints.

6. Conclusion

Colorectal cancer represents a growing burden on public health, particularly seen in a rise among younger adults. Early detection is crucial to reducing the mortality rate from this disease. Traditional colonoscopy remains the definitive diagnostic; however, its discomfort and inconvenience limit widespread patient acceptance.

Many current commercial designs have limitations with image quality, polyp detection, and data retrieval. The integration of high-definition camera and time-of-flight depth map imaging represents a meaningful improvement in diagnostic confidence compared to current two-dimensional devices. The use of wireless data transmission also eliminates physical capsule recovery which improves the patient's experience. The proposed device serves as an accessible, at-home screening tool, with the possibility of increasing the number of patients to engage with the early stages of colon cancer. Continued development of capsule endoscopy and clinical evaluation of such devices will be critical to fully capture the technological potential in colorectal cancer prevention. Clinical evaluation of such next-generation devices will be critical to realizing the full potential of accessible, at-home screening and achieving meaningful reductions in colorectal cancer mortality.

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How Does Chronic Academic Stress Affect The Neurobiological Disruption Of The PFC-Amygdala and The Hippocampus Circuitry By Rama Nashashibi

Abstract

Chronic academic stress is a state of persistent psychological distress that significantly changes the structure of the nervous system by disturbing the connectivity of the cortico-limbic triad formed by the prefrontal cortex (PFC), hippocampus, and amygdala. The purpose of this literature review is to assess the current neurobiological evidence that explains the structural remodeling of the PFC, hippocampus, and amygdala circuit induced by chronic academic stress. A database search performed among the four major online databases (PubMed, Google Scholar, PsycINFO, and Scopus) from June to July 2026 has resulted in the identification of 50 potentially relevant research papers. Thirty articles were excluded at the title screening stage, and 5 were excluded at abstract review, leaving 15 primary articles included in the analysis. The results of the included studies indicate that chronic exposure to high levels of glucocorticoids and catecholamines leads to extreme dendritic atrophy, decrease of synaptic spine density, and thinning of the gray matter in the PFC and CA1/CA3 subregions of the hippocampus, as well as reduced number of new neurons in the dentate gyrus, in contrast with dendritic hypertrophy and hyperexcitability in the basolateral amygdala (BLA). Such neuroanatomical imbalance creates an abnormal feedback loop in which a dysfunctional PFC and an impaired hippocampus fail to provide sufficient top-down inhibition to a hyperactive BLA, keeping the HPA axis in overdrive and causing executive dysfunction and academic burnout. Ultimately, these findings demonstrate that chronic academic stress drives significant neurostructural dysregulation, underscoring the critical need for institutional reforms that reshape academic environments to protect student neurocognitive health.

Introduction

Chronic academic stress is a persistent, long-term psychological state of pressure and strain resulting from exposure to academic stress over an extended period. This is in contrast to short-term academic stress, such as the nerves before a single exam. Chronic academic stress does not resolve after a single event, but carries on across weeks, months, and even years. Common sources of chronic academic stress include an unbroken heavy workload, high expectations, and time management struggles. During a period of chronic academic stress, the body's stress response system (e.g., cortisol, adrenaline, glucocorticoids, and noradrenaline) is repeatedly activated. This activation occurs due to inadequate recovery time, resulting in noticeable physical and emotional symptoms (Knezevic et al.,2023).

If left unaddressed, chronic academic stress and biological stress response can lead to academic burnout. This progression from prolonged stress to burnout is not merely behavioral or psychological; it is deeply rooted in physiological changes within the brain. Chronic exposure to stress hormones, such as cortisol, can significantly alter neural structures and disrupt cognitive functioning. Specifically, these prolonged states of neurological activation often impair regions

critical for executive function and self-regulation, making it essential to examine the role of the prefrontal cortex in mediating the academic experience (Shields et al., 2016).

The prefrontal cortex (PFC) is the anterior portion of the frontal lobe of the brain and plays a critical role in managing complex cognitive processes and regulating behavior, including those related to chronic academic stress (Friedman & Robbins, 2021). The PFC has several highly specialized subregions: the dorsolateral PFC (dlPFC), which handles working memory, task switching and problem solving; the orbitofrontal PFC (OFC), which evaluates risks, manages moral and social behavior; and the ventromedial PFC (vmPFC) which connects with emotional responses from the amygdala to facilitate logic allowing for empathy and personality expression (Friedman & Robbins, 2021). The PFC is one of the final regions of the brain to reach maturation (typically in the mid-to-late 20s), and its development plays a critical role in the transition from adolescence to adulthood (Arain et al., 2013). Therefore, any damage in this brain region can lead to challenges with emotional volatility and decision-making (Arnsten, 2009).

Another critical brain network is the limbic system. The limbic system is the network that is responsible for managing emotions, memory, and survival instincts (Rajmohan & Mohandas, 2007). The limbic system comprises several brain regions; the first is the amygdala, a cluster of nuclei located deep within the temporal lobe of the brain (Salzman,2026). The primary function of the amygdala is the processing of emotions, especially fear, anxiety, and aggression (Pessoa and Adolphs, 2010). The hippocampus is a second critical limbic structure and is a seahorse-shaped structure located deep in the temporal lobe of the brain. The hippocampus plays a central role in memory formation and spatial navigation (Chauhan et al., 2021). Dysregulation within these structures is often implicated in stress-related disorders, suggesting that the limbic system is highly sensitive to environmental stressors.

Having established the individual roles of the PFC and limbic system, it is important to consider how chronic stress affects them collectively. The aim of this review is therefore to identify specific domains that need further investigation to clearly understand the interaction between chronic stress and the triad of the PFC, amygdala, and hippocampus. Of interest are gender differences, the impact of age, and the translation of animal models to humans. Specifically, sex-dependent differences in HPA-axis reactivity alter stress vulnerability, while age influences baseline neural plasticity and structural resilience to chronic strain. Bridging animal stress paradigms with human academic data is also essential to ensure these biological findings translate accurately across broader populations.

Methods

To ensure a rigorous, transparent, and comprehensive analysis of the existing literature regarding the neurobiological impacts of chronic stress on the brain, a systematic search and testing protocol was established. A comprehensive literature search was conducted across four primary databases: PubMed, Google Scholar, PsycINFO, and Scopus in June and July 2026. The search strategy employed combinations of Boolean operators and free text keywords targeting the nature of the stressor, the target neuroanatomy, and the resulting biological or cognitive

outcomes. To ensure a comprehensive synthesis of the literature, search terms were categorized into distinct domain clusters representing the target population, physiological stress pathways, neuroanatomical regions, and functional and cognitive outcomes. Clustering search terms into specific domains is highly beneficial because this research question spans multiple biological scales from molecular hormone release to region-specific anatomical remodeling (PFC/hippocampal atrophy vs. amygdala hypertrophy) and behavioral outcomes. Clustering ensures full retrieval of granular cellular mechanisms while filtering out irrelevant non-academic stress literature. The search terms were identified using a domain clustering strategy designed to capture the multi-scalar nature of academic stress, spanning from molecular stress hormones to brain structure and cognitive performance. Search terms were grouped into five key categories: Target Population & Context (e.g., medical students, academic burnout), Physiological & Hormonal Markers (e.g., HPA axis, glucocorticoid sensitivity), Macro- Neuroanatomy (e.g., PFC, hippocampus, basolateral amygdala, dendritic atrophy), Functional and Cognitive Outcomes (e.g., executive dysfunction, working memory), Micro-Neuroanatomy and Neuromorphology, then combined using Boolean operators tailored to each database syntax to ensure comprehensive retrieval of cellular mechanisms while filtering out non-academic stress literature (Table 1).

Table 1. Search Strategy

Category	Primary Search Terms
Target Population & Context	Medical students, Stress in medical students, Academic stress, Academic burnout, Cognitive fatigue
Physiological & Hormonal Markers	HPA axis, Hypothalamic-Pituitary-Adrenal axis, Glucocorticoid receptor sensitivity, Chronic stress, Prolonged psychological stress
Macro-Neuroanatomy (Brain Regions)	Prefrontal cortex (PFC), Medial prefrontal cortex, Hippocampus, Dentate gyrus, Amygdala, Basolateral amygdala, Limbic system
Micro-Neuroanatomy & Neuromorphology	Prefrontal cortex atrophy, Dendritic atrophy, Amygdala hyperactivity, Amygdala hypertrophy, Basolateral amygdala hypertrophy, Hippocampus volume, Dendritic spine density
Functional & Cognitive Outcomes	Synaptic plasticity, Executive dysfunction, Working memory, Excitatory inhibitory balance, Limbic system dysregulation

Search strings were tailored to the syntax requirements of each database to ensure comprehensive coverage of the literature. Upon execution of the search strategy, all retrieved citations were gathered into a central database to prepare for eligibility screening and full-text assessment.

Inclusion Criteria & Exclusion Criteria

Articles retrieved from the database searches were filtered using strict, predefined criteria

to align with the aims of this review. To be included, papers had to feature either human or animal studies that focused specifically on chronic stress in general and academic stress in particular. Furthermore, selected papers were required to provide objective anatomical, neuroimaging, or biomarker data (specifically measuring cortisol levels) to substantiate their findings. Conversely, specific exclusion criteria were applied to narrow the scope of the data. Studies focusing solely on acute stress rather than chronic or academic stress were excluded from the sample. Additionally, animal-only studies were strictly excluded if they were not directly relevant to explaining human neurobiological mechanisms, ensuring that all synthesized animal models served a clear translational purpose.

The initial database search yielded a total collection of literature that was evaluated through two distinct phases of selection. First, a title and abstract examination was conducted to judge the direct relevance of the papers to the chronic stress framework and the neurobiological triad of the prefrontal cortex, amygdala, and hippocampus. Second, the remaining articles underwent a rigorous full-text evaluation to confirm they met all methodological benchmarks. Ultimately, the final sample of articles, comprising targeted human cohort studies paired with translationally relevant animal models, met all inclusion criteria and were selected for full thematic synthesis in this review.

Results

A total of 50 potentially relevant titles were identified and reviewed across different databases. Following title and abstract screening, 30 records were excluded for not meeting the relevance criteria for chronic academic stress and the PFC-amygdala-hippocampus circuit, leaving 20 articles for abstract review. Following abstract review, 5 articles were excluded for lacking objective anatomical, neuroimaging, or biomarker data, leaving 15 articles for full-text examination. All 15 full-text articles met inclusion criteria and were selected for inclusion in this review. Of the 50 records identified, 15 were retrieved from PubMed, 20 from Google Scholar, 10 from PsycINFO, and 5 from Scopus. Prolonged exposure to elevated stress leads to dendritic atrophy and reduced volume in both the PFC and the CA3/dentate gyrus regions of the hippocampus, resulting in impaired executive function, reduced working memory, suppressed adult neurogenesis, and diminished top-down emotional control. On the other hand, the basolateral amygdala exhibits hypertrophy and heightened reactivity, driving elevated baseline anxiety and persistent threat sensitivity. Together, this triad creates a dysregulated feedback loop where weakened top-down inhibition from a hypoactive PFC and structurally impaired hippocampus fails to restrict a hyperactive amygdala, maintaining cognitive deficits and emotional dysregulation. Methodologically, the included studies primarily employed high-resolution structural magnetic resonance imaging (MRI) alongside salivary cortisol assays to correlate prolonged stress exposure with circuit-level alterations.

Discussion

Academic Stress Effects on the Prefrontal Cortex

Chronic academic stress prompts structural changes, neurochemical imbalances, and cognitive and behavioral consequences in the PFC (Algaidi, 2025). Prolonged exposure to academic stressors induces significant structural remodeling within the PFC, dissimilar to acute stress, which causes temporary chemical changes; chronic academic stress changes the architecture of the PFC (Woo et al., 2021). Specifically, chronic academic stress has the potential to trigger dendrites in the PFC to shrink and retract, causing dendritic atrophy and resulting in physical changes in the PFC (Woo et al., 2021). Dendritic shrinkage and retraction in the PFC are problematic because they result in physical disconnection of neurons, which can weaken the neural circuits required for essential tasks like working memory and complex executive functions.

Along with dendritic shrinkage, chronic stress can result in a loss of dendritic spines, the small structures where synapses form. Fewer spines mean fewer connections, which translates to less efficient signal processing in the PFC, leading to reduced synaptic density. This is harmful because it leaves fewer communication pathways between neurons, making it harder for the brain to process information, learn, and form new memories (Woo et al., 2021). Furthermore, following chronic stress, several studies have found a reduction in the volume of gray matter (McEwen et al., 2016), which is the brain tissue made up of neuron cell bodies and synapses responsible for processing information in the central nervous system (Mercadante & Tadi, 2023). In the PFC, particularly the dlPFC and mPFC, there is a particularly high density of glucocorticoid receptors (the receptors cortisol binds to). The high concentration of glucocorticoid receptors makes the PFC more sensitive to chronic hypothalamic-pituitary-adrenal (HPA)-axis activation compared to other cortical areas (Packard et al., 2016). Therefore, chronic academic stress has the potential to disrupt critical neurochemical pathways in the PFC, primarily by provoking high levels of neurotransmitters like norepinephrine and dopamine, causing stress hormone increases in the PFC (Datta and Arnsten, 2019). The continuous elevation of these neurochemicals, while adaptive in the short term, fundamentally alters the cellular architecture of the prefrontal cortex over time. This biochemical shift disrupts the structural integrity of neurons, leading to the progressive retraction and remodeling of dendritic branches. Consequently, these structural changes provide a biological basis for the cognitive deficits observed in individuals experiencing prolonged academic pressure (McEwen et al., 2015).

Neurotransmitters initiated by chronic academic stress then activate feedforward signaling pathways (such as cyclic AMP and protein kinase C) that open potassium channels, essentially short-circuiting the electrical firing required for working memory (Arnsten, 2015). Behaviorally, this neurochemical imbalance manifests in the form of a substantial reduction in cognitive capacity; for example, students may have trouble focusing during lectures, frequently lose focus, often referred to as mind blanks, and find it more challenging to switch their attention between challenging academic tasks *(Diamond, 2013; Smallwood & Schooler, 2015)*. In addition to this pathway, chronic stress also disrupts the balance between the PFC's two primary

neurotransmitter systems - inhibitory gamma-aminobutyric acid (GABA) and excitatory glutamate - further compromising the network's ability to sustain firing. Therefore, stress causes an imbalance in excitatory and inhibitory neurotransmitters. This suppresses the ability of PFC neurons to hold information online, causing the PFC to become offline (Algaidi, 2025). Behaviorally, this PFC offline condition displays a pattern of greater distractibility, impulsive decision-making, and a reduced ability to control emotions. Because the brain's internal manager is essentially inactive, students may struggle to prioritize academic work, arrange projects, or follow multi-step instructions. This shift in neurochemical balance fundamentally alters the functional connectivity of the PFC, effectively decoupling it from other brain regions involved in executive function. As a result, the neural circuitry required for higher-level cognitive tasks becomes compromised, leading to the observable behavioral deficits.

The structural and chemical degradation of the PFC under chronic stress directly impairs higher-order cognitive processing, leading to deficits in working memory, cognitive flexibility, and attentional control (Girotti et al., 2024). Because PFC neurons can no longer sustain persistent firing to represent information, students often experience mind blanks and struggle with complex problem-solving. The architectural changes in the PFC make it difficult to shift attention between tasks (loss of cognitive flexibility). Students then have a more challenging time controlling impulses, staying focused, and suppressing inappropriate actions. Positively, these neuronal changes are generally not permanent. If the chronic stress stops, the brain can regrow dendrites and synapses because it has remarkable structural plasticity. Interventions such as regular breaks, getting adequate sleep, mindfulness, and physical exercise can help reduce cortisol levels and restore top-down control of the PFC (Arnsten, 2015). Importantly, the PFC does not operate in isolation from the hippocampus. Chronic stress leads to significant structural changes in the prefrontal cortex, which directly impair cognitive and working memory performance (Figure 1).

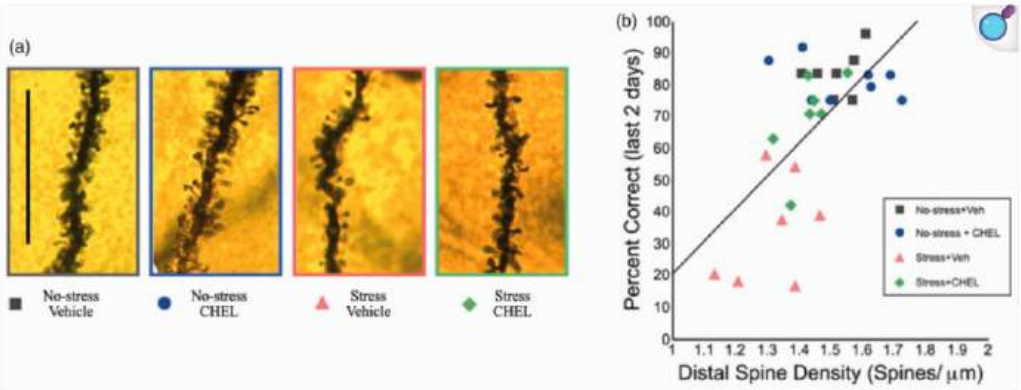


Figure 1. The impact of chronic stress on dendritic spine density and working memory. This figure shows the correlation between chronic stress, dendritic spine density, and working memory performance. (a) Representative images of rodent layer II/III prelimbic cortex showing reduced spine density under chronic stress. (b) Working memory performance correlated with distal spine density ($r = 0.636$, $P < 0.001$). From "Chronic Stress Weakens Connectivity in the Prefrontal Cortex," by E. Woo, L. H. Sansing, A. F. T. Arnsten, & D. Datta, 2021, *Chronic Stress*, 5, article 24705470211029254. Figures (a) and (b) adapted from Hains et al. (2009).

Academic Stress Effects on Hippocampus

Similar to its effects on the PFC, long-term exposure to high levels of glucocorticoids induces severe structural regression within the hippocampus (Kim and Diamond, 2002). The hippocampus is highly sensitive to stress due to its high density of glucocorticoid receptors. Chronicity of academic stress can trigger substantial dendritic atrophy. Specifically, shrinkage and retraction of dendrites often occur in CA3 and CA1 pyramidal neurons (McEwen et al.2016). The dendrites of hippocampal neurons, particularly in the CA3 region, shrink and pull back, resulting in a physical loss of synaptic connections (Kim et al.,2015). This loss of synaptic connections is highly detrimental because it directly impairs the hippocampus's ability to process and store new information, ultimately leading to deficits in learning, memory retention, and spatial navigation.

Additionally, cortisol from chronic stress halts the production of new neurons in the dentate gyrus, impairing the brain's natural ability to form new spatial and declarative memories (Kim et al., 2015). Over time, these combined effects can lead to an actual reduction in overall hippocampal volume, which is often subjectively experienced as brain fog, burnout, and impaired recall.

These structural changes can directly impair a student's ability to study and retrieve information because the hippocampus is the centre for consolidating short-term experiences into long-term declarative memory(Kim & Diamond, 2002; Squire, 1992). The structural degradation of CA1 and CA3 neurons severely affects Long-Term Potentiation (LTP), the cellular mechanism underlying learning and memory formation (Chan et al., 2007; Wheal et al., 1998), and the process of strengthening neural connections necessary for forming new memories. At the molecular level, this structural degradation disrupts synaptic plasticity by altering N-methyl-D-aspartate (NMDA) receptor function(Bliss and Collingridge, 1993; Tsien et al., 1996). Under non-stress conditions, normal NMDA receptor signalling facilitates LTP; however, chronic elevation of glucocorticoids causes an excessive influx of glutamate, leading to overstimulation of NMDA receptors (excitotoxicity) and a subunit shift in these receptors. This shift impairs LTP in the CA1 and CA3 regions of the hippocampus while enhancing Long-Term Depression (LTD), the systemic weakening of synaptic connections. Consequently, the brain's ability to consolidate complex academic information is suppressed, directly manifesting as learning difficulties and memory recall deficits under high academic pressure (Chen et al.,2025). Without functional LTP and healthy neurogenesis in the dentate gyrus, students struggle with declarative memory deficits, impaired pattern separation, and cognitive rigidity (Yun et al., 2023).

Academic Stress Effects on Amygdala

In contrast to the shrinkage and atrophy of structural atrophy in the prefrontal cortex and hippocampus induced by chronic academic stress, dendritic hypertrophy and synaptogenesis are prolonged, accompanied by persistent structural changes in the BLA (Vyas et al., 2002). The dendritic expansion and spine density increase as a consequence of sustained stress-induced

glucocorticoids lead the BLA pyramidal neurons to become hyperexcitable and hyperresponsive (Roozendaal et al., 2009). Importantly, whereas the dendritic shrinkage observed in the prefrontal cortex and hippocampal CA3 is readily remodeled and recovers following cessation of the stressor, the hypertrophy of dendrites within the BLA remains much longer, even outlasting the period of acute academic stress (Vyas et al.,2002; Roozendaal et al., 2009).

This persistent structural expansion severely impairs the interaction of the amygdala with other structures in stress circuits. The top-down inhibition provided by the prefrontal cortex to the amygdala in physiological states becomes impaired, as damage to the prefrontal cortex and BLA hypereactivity may create a positive feedback loop leading to overactivation of the HPA axis, hence increased susceptibility to academic threats (Kredlow et al., 2021). In the BLA, dendritic growth increases synaptic connections, making neurons more likely to fire. These structural growths leave the amygdala overexcited, thus increasing the basal levels of anxiety and enhancing its response to academic threatening situations such as deadlines and examinations (Vyas et al., 2006). The amygdala signals the HPA axis to release a stress hormone called cortisol whenever the person experiences a threat. Chronic stress leads to a hyperexcitable amygdala and overstimulation of the HPA axis, causing elevated levels of cortisol to be released (McEwen &Akil, 2020; Herman et al., 2016).

The PFC sends a top-down inhibitory signal that quiets the amygdala. In cases of chronic stress, this process is undermined when shrinking prefrontal neurons lose contact with hyperactive BLA neurons, thus causing the amygdala to remain overactive, prolonging anxiety and stress. When someone is calm, the PFC and hippocampus provide them with feedback inhibition to their HPA axis to decrease the production of cortisol as soon as the threat passes. The amygdala, however, stimulates this feedback in a positive loop, thereby increasing cortisol production in the presence of stress. Chronic academic stress damages inhibitory feedback circuits, such as the PFC and the hippocampal CA3 area, and enhances excitatory feedback circuits, such as the BLA, leading to uninhibited cortisol release (Figures 2 and 3).

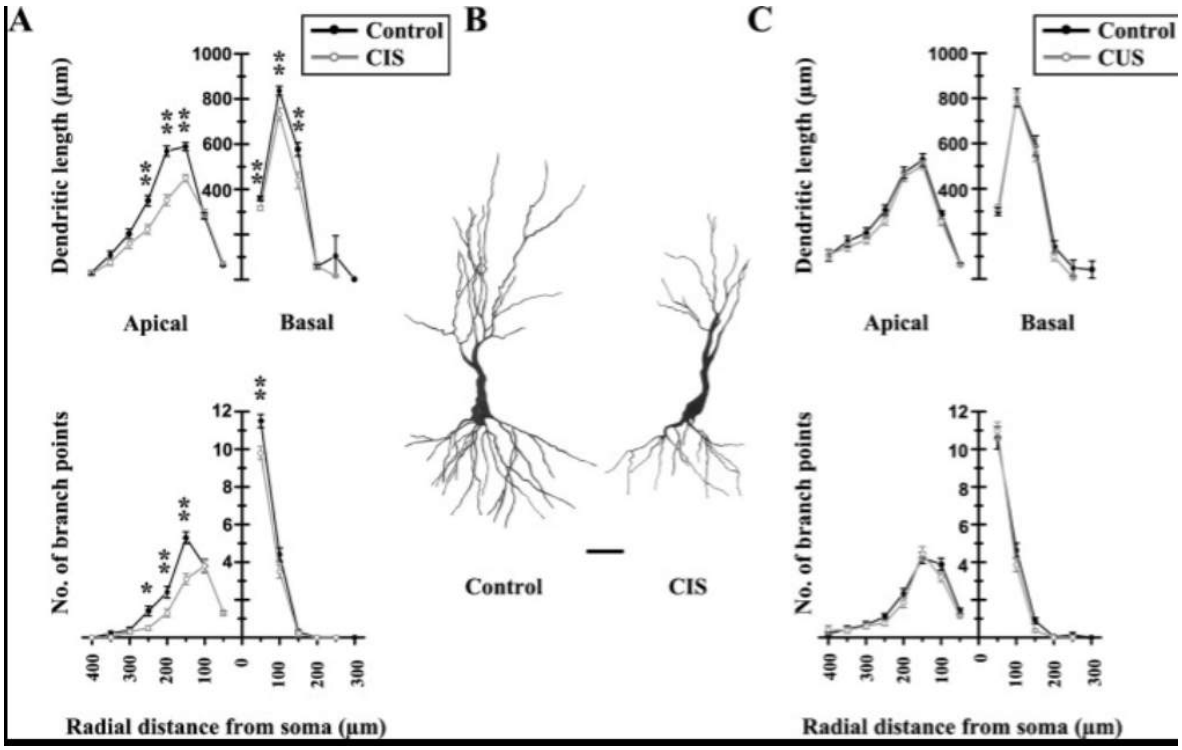


Figure 2. Chronic stress shrinks brain cells in the hippocampus (Vyas et al., 2002). *(A)* Graphs showing a significant decrease in the length and branching of dendrites (cell branches) in stressed rats (CIS; open circles) compared to non-stressed rats (Control; black circles). *(B)* Microscopic tracings of a normal hippocampal neuron (Control) versus a shrunk neuron after chronic stress (CIS). *(C)* Similar shrinking effects shown under chronic unpredictable stress (CUS). Asterisks (*) show where the difference between groups is statistically significant.Adapted from Vyas et al. (2002).

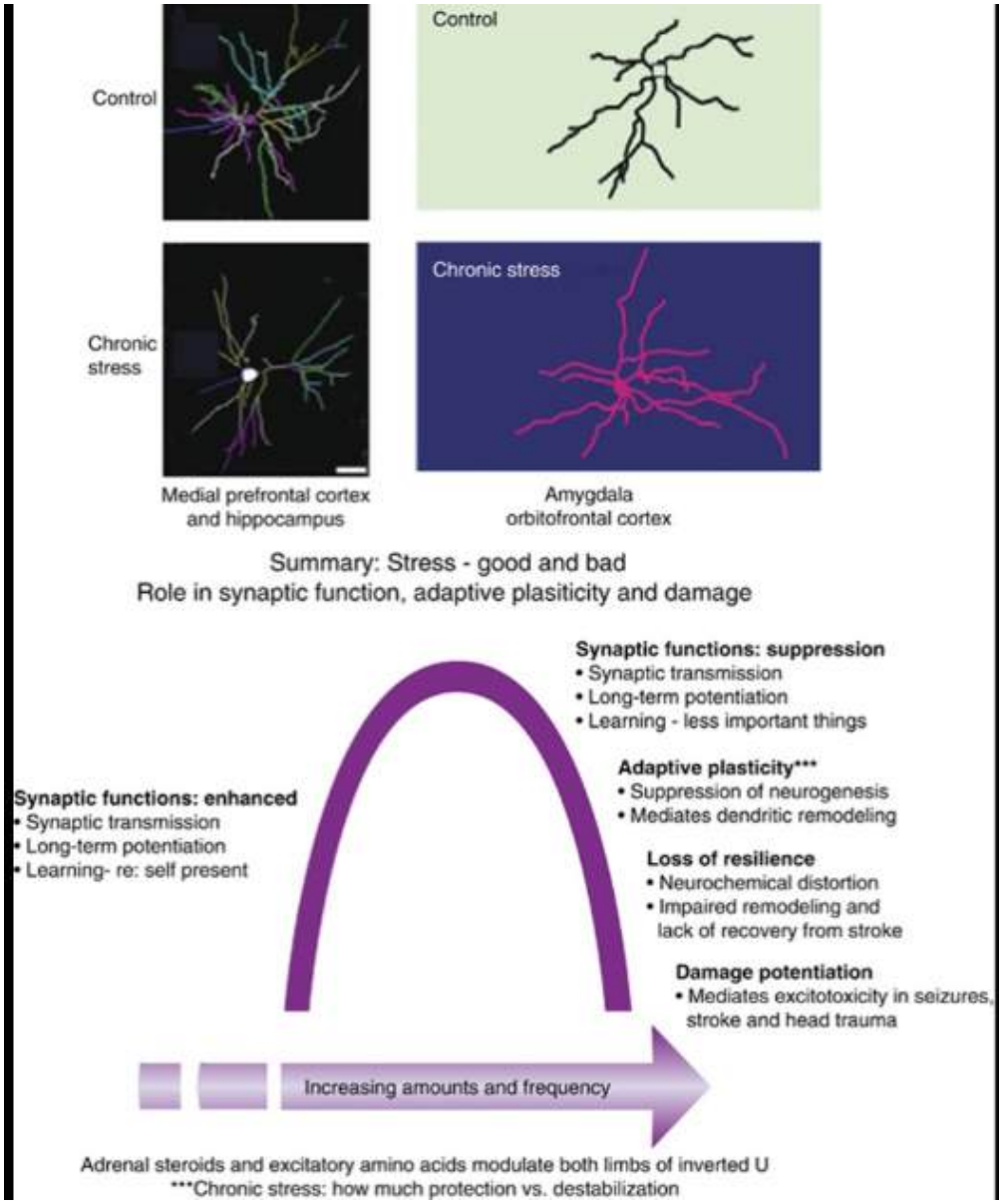


Figure 3. Structural dendritic remodeling and non-linear synaptic responses to chronic stress across cortico-limbic networks. *(Top)* Representative digital reconstructions comparing neuronal morphology in control versus chronic stress conditions. Neurons in the medial prefrontal cortex (mPFC) and hippocampus exhibit marked dendritic atrophy, retraction, and spine loss following chronic stress exposure. Conversely, neurons in the basolateral amygdala and orbitofrontal cortex (OFC) undergo dendritic hypertrophy, demonstrating increased arborization and spine density.

(Bottom) The non-linear (inverted-U) relationship between stress levels/frequency and neural plasticity. Moderate, acute stress facilitates adaptive plasticity, long-term potentiation (LTP), and synaptic transmission. In contrast, chronic or severe stress shifts the dynamic toward maladaptive outcomes, including the suppression of adult neurogenesis, neurochemical distortion, impairment of structural recovery, and heightened susceptibility to excitotoxic damage. *(Adapted from McEwen et al., 2016).*

The Neurobiological Impact of Chronic Academic Stress

Beyond cellular damage, chronic stress fundamentally disrupts inter-regional communication. This breakdown weakens top-down executive control, forcing a shift from balanced cognition to reactive, fear-based processing. Under non-stress conditions**,** the PFC, the hippocampus, and the amygdala form a highly organized and coordinated network. In this balanced condition, the PFC and the hippocampus exert an essential inhibitory control over the amygdala, keeping emotional and fear responses under control and ready only when needed (McEwen et al., *2016*). However, chronic academic stress thoroughly disrupts this balanced circuitry. Extended periods of elevations of cortisol and catecholamines essentially weaken the structural integrity of both the PFC and the hippocampus, ruining their capacity to suppress the amygdala. Stripped of this inhibition, the amygdala undergoes dendritic hypertrophy, becoming hyperactive. This functional decoupling shifts the brain from thoughtful, goal-directed regulation to a state of reflexive, fear-driven anxiety, locking the student into a persistent fight-or-flight loop that severely impairs both academic learning and emotional stability (McEwen & Morrison, 2013; Vogel & Schwabe,2016). This trend illustrates how the duration and intensity of the stimulus have a significant impact on the brain's reaction to stress. Although the hippocampus is initially resilient, its ability to adapt is limited, and switching from acute to chronic stress exposure may result in dysfunctional rather than beneficial effects. Determining the molecular mechanisms by which extended environmental stresses affect cognitive health requires an understanding of these dynamics (Figure 4).

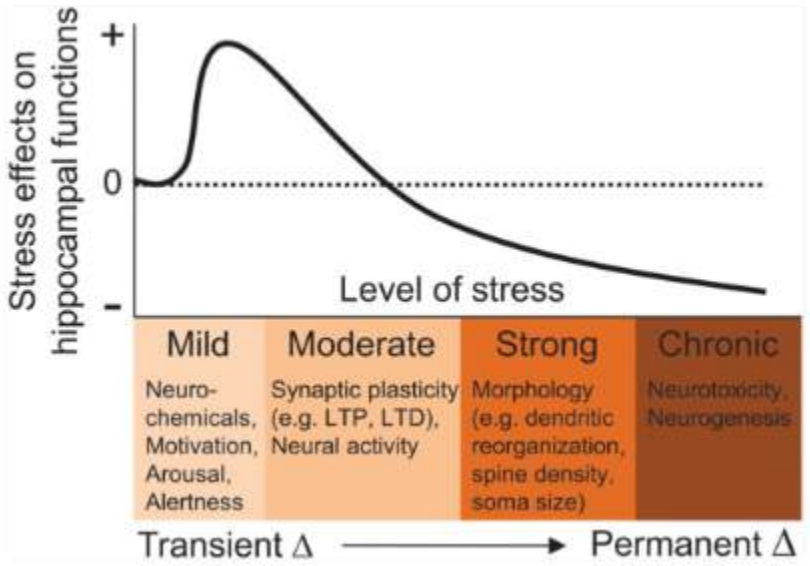


Figure 4. The inverted U-shaped relationship between stress severity and hippocampal function. This figure shows an inverted U-shaped relationship between stress severity and hippocampal structure and function. Mild, acute stress promotes temporary adaptive enhancements in neurochemistry, alertness, and motivation. As stress severity increases to moderate and strong intensities, synaptic plasticity (LTP/LTD) deteriorates, and morphological remodeling (such as reduced spine density and dendritic reorganization) occurs. Under chronic stress, these changes cross an adaptive threshold, resulting in permanent neurotoxic damage and suppressed neurogenesis. Adapted from McEwen (2007) and Joëls et al.(2006)

Strengths

This literature review possesses several key methodological and conceptual strengths that enhance its contribution to the understanding of stress-induced neuroplasticity. First, the review uses a rigorous and transparent selection protocol that reduces 50 database search results to 15 primary studies, requiring objective neuroimaging or biomarker evidence (e.g., cortisol metrics and structural MRI data) strictly. This methodological filter removes self-report bias and provides high empirical validity to the synthesized findings (Dorsey et al., 2022). This is a more integrative network-level synthesis of the cortico-limbic triad than regional analyses, explaining how differential structural effects (dendritic atrophy in the prefrontal cortex and CA1/CA3 hippocampal subregions, and dendritic hypertrophy in the basolateral amygdala) lead to a breakdown in top-down inhibition and the maintenance of HPA-axis overdrive. This synthesis provides a biologically plausible and contextually relevant framework for academic stress research through the integration of translational animal models with human cohort data and the framing of structural remodeling with functional cognitive outcomes.

Limitations

While this review provides the necessary information on the effects of academic pressure in the PFC, hippocampus, and amygdala, and provides a comprehensive overview of how chronic academic stress disrupts neurobiological processes in the PFC-amygdala and hippocampal circuitry, it is important to consider these results in the context of several limitations. First, there is a lack of literature explicitly examining academic stressors in humans. Therefore, the research utilized here uses animal models of chronic stress to understand long-term effects of academic stressors. In addition, the existing human studies are limited to cross-sectional assessment of academic stress, which prevents researchers from establishing causal links between academic stress and neural changes. Finally, the field lacks generalizability due to its overreliance on highly specific, homogeneous samples, e.g., medical and law students, who often possess unique baseline cognitive reserves and socioeconomic advantages that do not reflect the broader, more diverse student population.

Future Directions

There are several important future directions to consider. First, future research must shift toward longitudinal human neuroimaging designs that track brain development before, during, and after periods of intense academic pressure to establish a clear developmental path of neural change. These studies should be paired with intervention and reversibility trials to determine whether stress-induced neural changes can be undone, while carefully isolating academic stress from confounding chronic stressors. Crucially, future inquiries must investigate how chronic versus acute academic stress alters functional connectivity between the amygdala and hippocampus during critical developmental periods, while prioritizing the understudied area of sex differences in HPA-axis reactivity and stress hormone sensitivity. Researchers should also expand participant pools to include larger, highly diverse demographic groups to evaluate how

socioeconomic status, school climate, sleep deprivation, and social support act as moderating factors. Ultimately, this work must translate into practical applications by exploring whether long-term working memory training can drive protective transfer effects, effectively rewiring the brain for resilience, and how specific instructional techniques can be formally integrated into school curricula to serve as a structural buffer against academic stress.

Furthermore, longitudinal study designs will be important to track the development of these neurobiological adaptations over time, providing major insights into whether early interventions during critical periods of development can prevent long-term cognitive deficits. We need interdisciplinary collaborations among neuroscientists, educators, and policymakers to translate laboratory findings into real-world application. Such partnerships will not only make neuroscientifically based interventions practical and culturally adaptable across various school contexts but will also help inform policy changes that address the systemic causes of academic burnout before it occurs at a neural level.

Implications

Beyond individual-level interventions, these findings highlight the need for systematic changes in educational frameworks. By moving away from a primary focus on high-stakes testing and toward learning environments that prioritize student mental well-being, educational systems can help reduce the chronic stress that affects the adolescent brain. Moving forward, it is essential to implement school-based programs that balance academic rigor with support for cognitive health, ensuring that the drive for achievement does not come at the cost of healthy neurological development.

Conclusion

In conclusion, this review identifies the impacts of chronic academic stress on the brain. Specifically, chronic academic stress, driven by sustained cortisol elevation, produces region-specific structural changes. The PFC shows reduced gray matter volume and dendritic atrophy, impairing executive function. The hippocampus exhibits reduced volume and suppressed neurogenesis, impairing memory and disrupting cortisol regulation via the HPA axis. The amygdala undergoes dendritic hypertrophy and increased activity, heightening emotional reactivity. In sum, academic stress leads to a disruption of the PFC, hippocampal, and amygdala circuitry, resulting in difficulty remembering studied material and causing deterioration in academic performance. Chronic academic stress rewires the brain, structurally weakening the centers for executive control and memory while hyperactivating fear circuits, creating a self-perpetuating biological loop of cognitive decline and emotional exhaustion.

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A Meta-Analysis of Dysautonomia and the Prevalence of Autonomic-Type Symptoms Among High School Students By Benay Ozcelik

ABSTRACT

Dysautonomia is a group of conditions in which the autonomic nervous system (ANS) does not function properly. The ANS controls involuntary functions such as heart rate, blood pressure, breathing, and body temperature regulation, and ANS dysfunction disrupts these processes. Adolescents are one of the most commonly affected populations in these conditions, however there is limited research on how dysautonomia-associated symptoms appear in high-stress, competitive academic environments. This study aims to address this gap by investigating the prevalence of self-reported autonomic-type symptoms and assessing student awareness of dysautonomia at Seven Lakes High School (SLHS), where students face significant academic pressure.

A 29-question anonymous survey was administered to students at Seven Lakes High School (SLHS) in Katy, Texas, in April 2026. The survey, which was reviewed and approved by the school's Health Occupations Students of America (HOSA) faculty advisor prior to distribution, assessed 13 symptoms from a dysautonomia-associated symptom checklist, along with lifestyle habits, self-reported stress levels, and awareness of the condition. Importantly, this study measured only self-reported symptom burden; it does not establish clinical diagnoses of dysautonomia or any related disorder.

There were 404 respondents. Results indicated that 62.6% of students experienced lightheadedness or dizziness upon standing, and 48.5% reported difficulty concentrating (brain fog). Female students reported significantly higher symptom burdens than male students (mean = 4.81 vs. 2.85; $p < 0.001$, Cohen's $d = 0.74$), and students reporting joint hypermobility had higher symptom counts than their peers (mean = 5.06 vs. 4.05; $p = 0.003$). Perceived stress showed the strongest association with symptom burden and remained the strongest independent predictor in the multiple regression model ($r = 0.413$, $p < 0.001$). A multiple regression analysis ($R^2 = 0.230$) identified stress, female sex, joint hypermobility, regular exercise, and grade level as significant independent predictors of autonomic symptoms. Furthermore, autonomic symptoms increased steadily as the number of reported risk factors increased.

Despite the high prevalence of self-reported autonomic-type symptoms, 94.3% of students reported a lack of knowledge about dysautonomia, and 78.2% were unaware of simple, evidence-based techniques to manage orthostatic symptoms. These findings show that dysautonomia-associated symptoms are commonly reported among the students at SLHS, while awareness of the condition remains very limited. It is important to note that these symptoms can easily be mistaken for other common issues like anxiety, anemia, or sleep deprivation; therefore, proper medical evaluation is necessary to distinguish true ANS dysfunction from other possible causes. Increasing student awareness and encouraging appropriate medical evaluation may help identify students who could benefit from further assessment.

Keywords: autonomic dysfunction; dysautonomia; POTS; teenagers; adolescents; stress; joint hypermobility; orthostatic intolerance; high school health; symptom prevalence

INTRODUCTION

The autonomic nervous system (ANS) regulates the involuntary body functions such as heart rate, blood pressure, breathing, digestion, and body temperature. Autonomic dysfunction, also known as dysautonomia occurs when the ANS fails to function properly.[1]Diagnosing dysautonomia can be challenging because many of its symptoms including lightheadedness, dizziness, fatigue, brain fog, and palpitations occur in some other common conditions such as anxiety, anemia, dehydration, migraine, and sleep deprivation. In the long term, chronic autonomic dysfunction can significantly affect the quality of life and often lead to persistent physical deconditioning, occupational or academic impairment, and secondary psychological distress such as chronic anxiety or depression.[2] Chronic burden of severe fatigue, cognitive lapses (brain fog), and exercise intolerance frequently lead to substantial educational disruption, social isolation, and professional underemployment or disability.[3] Therefore, delay in diagnosing this condition is common with an average time from symptom onset to diagnosis above five years.[4,5]

The underlying neurobiology for this condition involves dysregulated autonomic signaling, characterized by inappropriate sympathetic surges (such as high norepinephrine release leading to palpitations), paired with parasympathetic deficits. Pathophysiologically, disorders like Postural Orthostatic Tachycardia Syndrome (POTS) are often rooted in hypovolemia, small fiber neuropathies, or receptor targeted autoantibodies affecting adrenergic and muscarinic G-protein coupled receptors.[6] Genetically, these disorders range from polygenic and connective tissue linked forms to monogenic conditions like familial dysautonomia, which is driven by ELP1 mutations that compromise autonomic and sensory neuron survival.[7]

There are various forms of dysautonomia. One of the most common forms in adolescents is POTS, which is characterized by a significant increase in heart rate upon standing. Other common symptoms include dizziness, visual disturbances, brain fog, fatigue, and, in some cases, fainting (syncope).[8] POTS affects approximately 1–3 million Americans, and most commonly develops during adolescence, with females affected four to five times more often than males.[9-11] Joint hypermobility, commonly described as being "double-jointed," is another risk factor for dysautonomia. Increased connective tissue flexibility may reduce the body's ability to maintain blood pressure while standing, contributing to orthostatic symptoms.[12] These symptoms can interfere with concentration, memory, note-taking, and classroom participation, making them especially relevant for students.[13]

Despite increasing recognition of dysautonomia, there is limited research which examined the prevalence of dysautonomia-associated symptoms in high school students or evaluated their awareness of the condition. No published study has systematically surveyed a high school population to examine the prevalence of dysautonomia-associated symptoms,

identify factors associated with greater symptom burden, and to assess awareness of dysautonomia and available self-management strategies.

This study investigated the prevalence of self-reported dysautonomia-associated symptoms, factors associated with symptom burden, and student awareness of dysautonomia in a high school population. This was not a diagnostic study and self-reported symptoms cannot establish a clinical diagnosis of dysautonomia. Many of the symptoms in the survey may also result from other medical conditions commonly seen in adolescents. Therefore, this study was not designed to determine how many students have dysautonomia.

METHODS

Study Design and Setting

This cross-sectional survey study was conducted in April 2026 at Seven Lakes High School (SLHS), a large public high school in Katy, Texas, serving students in grades 9–12. The school is recognized for its strong academic performance, STEM programs, and high college readiness.

Survey Instrument

A 29-question survey was developed for this study and administered using Google Forms. The survey included six sections: (1) demographics (grade level, gender, and self-reported joint hypermobility); (2) a 13-item checklist of symptoms commonly associated with dysautonomia; (3) symptom frequency rated on a 5-point scale (1 = never, 5 = daily or always); (4) lifestyle factors (daily water intake, nightly sleep duration, and regular exercise); (5) perceived stress rated on a 5-point scale; and (6) awareness of dysautonomia, knowledge of physical counter-maneuvers, and interest in learning more about the condition. The complete survey is provided in Supplementary Appendix A. The symptom checklist was developed from published clinical descriptions of dysautonomia and POTS but was not a validated diagnostic instrument. To reduce response bias, students completed the symptom questions before being informed that the survey focused on dysautonomia.

Recruitment and Ethics

The survey was reviewed and approved by the HOSA faculty advisor of SLHS, and the survey link was distributed by HOSA advisor, HOSA members and teachers through HOSA club communications and school-approved channels. Participation was voluntary and anonymous, and no personal identifying information was collected. Formal Institutional Review Board (IRB) review was not obtained because the study met the criteria for exemption applicable to anonymous educational survey research under the Code of Federal Regulations (45 CFR §46.101(b)(2)).[14]

Statistical Analysis

The data was analyzed using IBM SPSS Statistics (Version 29).[15] The primary outcome measure was total symptom count (range: 0–13). The symptom burden was analyzed across seven predefined groupings including the overall study population, gender, joint hypermobility, perceived stress level, regular exercise, daily sleep duration and water intake.
Symptom prevalence for each symptom was calculated using binary responses (present vs. absent). Chi-square (χ^2) test was used to compare symptom prevalence between groups. Independent samples *t*-tests and one-way analysis of variance (ANOVA) compared mean symptom counts. Pearson correlation examined the relationships between continuous variables and symptom count, and multiple linear regression analysis was used to identify independent factors associated with symptom burden. Statistical significance was defined as $p < 0.05$.

RESULTS

1. Participant Demographics and the Prevalence of Autonomic-Type Symptoms

A total of 404 students participated in the survey. Demographic characteristics are summarized in Table 1. Approximately 71% of the respondents were female, most respondents were in 9th or 10th grade (75.3% combined), and approximately 30% of the respondents self-reported to be double jointed.

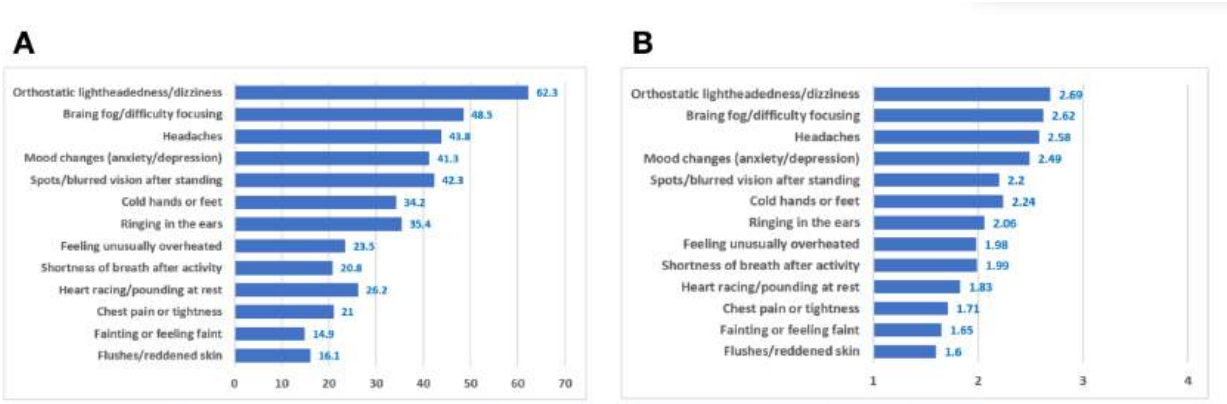
Table 1. Participant Demographics. There are three columns with characteristics showing the list of selected parameters and n is for the number of participants and % is for percentage.

Characteristic	n	%
GENDER		
Female	286	70.8%
Male	110	27.2%
Prefer not to respond	8	2.0%
GRADE LEVEL		
9th Grade	136	33.7%
10th Grade	168	41.6%
11th Grade	80	19.8%
12th Grade	20	4.9%
JOINT HYPERMOBILITY (Self-Reported)		
Yes	119	29.5%
No	249	61.6%
Not sure / N/A	36	8.9%

Note. n: number of participants, %: percentage of participants. Joint hypermobility was assessed by single-item self-report, not a validated clinical instrument (e.g., Beighton Score).

The prevalence and mean frequency of the symptoms are shown in Figure 1A, 1B, 1C.. The most commonly reported symptom was orthostatic lightheadedness/dizziness (62.6%), followed by brain fog (48.5%). Headaches, mood changes, and visual disturbances each affected

more than 40% of respondents. Fainting was reported by 14.9% of the students. The mean symptom count was 4.31±2.99, and 68.6% of the students reported three or more symptoms.



C

Symptom	n	%	Mean Intensity (1–5)*
Orthostatic lightheaded/dizziness	253	62.6%	2.69
Brain fog / difficulty focusing	196	48.5%	2.62
Headaches	177	43.8%	2.58
Mood changes (anxiety/depression)	167	41.3%	2.49
Spots/blurred vision after standing	171	42.3%	2.20
Cold hands or feet	138	34.2%	2.24
Ringing in the ears	143	35.4%	2.06
Feeling unusually overheated	95	23.5%	1.98
Shortness of breath after activity	84	20.8%	1.99
Heart racing/pounding at rest	106	26.2%	1.83
Chest pain or tightness	85	21.0%	1.71
Fainting or feeling faint	60	14.9%	1.65
Flushed/reddened skin	65	16.1%	1.60
Overall mean symptom number: 4.31± 2.99; 68.6% reported ≥3 symptoms			

Figure 1. Prevalence of Autonomic-Type Symptoms. A. The X-axis shows the prevalence of each symptom in percentage in the whole study population. The Y-axis shows the list of the symptoms. **B.** Shows the mean Symptom Intensity. The X-axis shows the intensity that the students experienced the symptoms. The intensity scale: 1 = never, 2 = rarely, 3 = sometimes, 4 = often, 5 = daily or always. The Y-axis shows the list of the symptoms. **C.** Combined view of prevalence and mean intensity for symptoms. Note for the Table. n: number of participants. %: percentage of the symptoms reported by the participants. Mean intensity: the intensity of the symptoms reported by participants *Intensity scale: 1 = never, 2 = rarely, 3 = sometimes, 4 = often, 5 = daily or always.

2- The female students reported significantly higher mean symptom

Comparison of symptom prevalence in genders is shown in Table 2. The female students reported significantly higher mean symptom numbers compared to the male students (4.81±2.98 vs 2.85±2.44, respectively; $p < 0.001$). Eleven of the 13 symptoms were found to have significantly higher prevalence in female students with the highest prevalence seen in mood changes, followed by cold hands/feet and heart racing/pounding.

Table 2. Autonomic-Type Symptom Prevalence by Gender.

Symptom	Female (n=286)	Male (n=110)	p-value
Orthostatic lightheaded/dizziness*	66.1%	53.6%	0.032
Brain fog / difficulty focusing	51.4%	39.1%	0.051
Headaches*	47.6%	31.8%	0.014
Mood changes (anxiety/depression)***	50.7%	17.3%	<0.001
Spots/blurred vision after standing**	46.2%	29.1%	0.004
Cold hands or feet***	42.7%	13.6%	<0.001
Ringing in the ears**	40.2%	21.8%	0.006
Feeling unusually overheated**	28.7%	10.9%	0.003
Shortness of breath after activity***	26.9%	5.5%	<0.001
Heart racing/pounding at rest***	34.3%	8.2%	<0.001
Chest pain or tightness***	25.9%	8.2%	<0.001
Fainting or feeling faint***	19.2%	3.6%	<0.001
Flushed/reddened skin***	21.7%	2.7%	<0.001

*Note. n: number or participants. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.*

3- Symptom count was significantly high in double-jointed students

Symptom prevalence by joint hypermobility is shown Table 3. Thirty-two students who did not answer the hypermobility question on the survey were not involved in the statistical analysis. Mean symptom count was significantly high in double-jointed students (who self-reported to have joint hypermobility in her/him-self or in the family members) compared to the non-double-jointed ones (5.06±3.01 vs 4.05±2.90, respectively, $p = 0.003$). Seven of 13 symptoms were significantly more prevalent in the hypermobile group. Cold hands/feet, and flushed/reddened skin were reported significantly higher in the hypermobile group.

Table 3. Autonomic-Type Symptom Prevalence by Joint Hypermobility.

Symptom	Hypermobile (n=119)	Non-Hypermobile (n=249)	p-value
Orthostatic lightheadedness/dizziness	68.1%	59.8%	0.130
Brain fog / difficulty focusing	54.6%	45.4%	0.115
Headaches	48.7%	41.4%	0.193
Mood changes (anxiety/depression)	48.7%	37.8%	0.055
Spots/blurred vision after standing*	51.3%	38.6%	0.021
Cold hands or feet**	46.2%	28.5%	0.002
Ringing in the ears*	42.9%	31.3%	0.046
Feeling unusually overheated*	31.9%	19.7%	0.016
Shortness of breath after activity*	27.7%	17.3%	0.038
Heart racing/pounding at rest*	34.5%	22.1%	0.016
Chest pain or tightness	24.4%	19.3%	0.291
Fainting or feeling faint	18.5%	13.3%	0.208
Flushed/reddened skin**	25.2%	12.0%	0.001

*Note. n: number of participants. * $p < 0.05$; ** $p < 0.01$. Joint hypermobility was assessed by a single-item self-report, not a validated clinical assessment.*

4- Prevalence of each symptom increased significantly in high stress levels

Figure 3A shows the comparison of the symptom prevalence across the three stress levels (Low, Moderate, and High), and demonstrates the trends of the symptom (Figure 3B) prevalence by stress levels. The findings showed that the prevalence of each symptom increased significantly in high stress levels (mean symptom counts: 2.63±2.28 in Low, 4.10±2.73 in Moderate, and 5.79±3.17 in High stress group; $p < 0.001$). The findings indicate that the higher the stress level, the more common the symptoms become. The broader correlations between stress level and autonomic-Type symptoms were presented in Figure 3C.

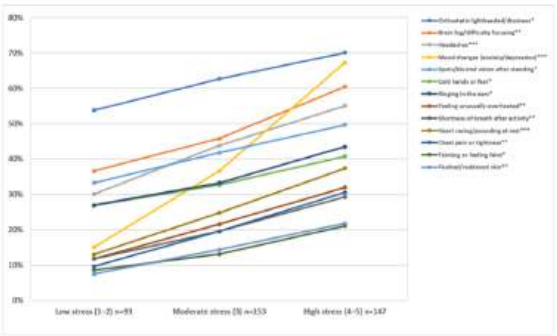
A

Symptom	Low Stress (1–2) n=93	Moderate Stress (3) n=153	High Stress (4–5) n=147	p-value
Orthostatic lightheaded/dizziness*	53.8%	62.7%	70.1%	0.016
Brain fog/difficulty focusing**	36.6%	45.8%	60.5%	0.002
Headaches***	30.1%	43.8%	55.1%	<0.001
Mood changes (anxiety/depression)***	15.1%	36.6%	67.3%	<0.001
Spots/blurred vision after standing*	33.3%	41.8%	49.7%	0.024
Cold hands or feet*	26.9%	32.7%	40.8%	0.049
Ringing in the ears*	27.0%	33.3%	43.5%	0.018
Feeling unusually overheated**	11.8%	21.6%	32.0%	0.004
Shortness of breath after activity**	11.8%	19.6%	29.3%	0.004
Heart racing/pounding at rest***	13.0%	24.8%	37.4%	<0.001
Chest pain or tightness**	9.7%	19.6%	30.6%	0.002
Fainting or feeling faint*	8.6%	13.1%	21.1%	0.026
Flushed/reddened skin**	7.5%	14.4%	21.8%	0.009

C

Symptom	r	p-value
Mood changes (anxiety/depression)	0.501	<0.001***
Headaches	0.368	<0.001***
Heart racing/pounding at rest	0.355	<0.001***
Brain fog/difficulty focusing	0.301	<0.001***
Fainting or feeling faint	0.296	<0.001***
Shortness of breath after activity	0.280	<0.001***
Flushed/reddened skin	0.275	<0.001***
Chest pain or tightness	0.271	<0.001***
Orthostatic lightheadedness/dizziness	0.260	<0.001***
Spots/blurred vision after standing	0.239	<0.001***
Feeling unusually overheated	0.237	<0.001***
Ringing in the ears	0.191	<0.001***
Cold hands/feet	0.126	0.017*

B



D

Stress Level	n	Mean Symptom Counts±SD	% with ≥3 Symptoms
1 — Very Low	27	2.19±2.06	33.3%
2 — Low	66	2.83±2.53	43.9%
3 — Moderate	153	4.10±2.62	71.2%
4 — High	124	5.13±2.80	83.9%
5 — Very High	**23**	**7.83±3.60**	**87.0%**

Figure 3: Trend of Autonomic-Type Symptom Prevalence by Stress Level. A. Autonomic-Type Symptom Prevalence Across the Three Stress Levels. The first column shows the list of the symptoms. The columns 2nd-4th show the stress levels (low, moderate and high stress). The numbers in parentheses show the stress level: Low stress (stress level 1 and 2), moderate stress (stress level 3) and high stress (stress level 4 and 5). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. **B.** Trend of Autonomic-Type SymptomS. The x-axis shows the level of the stress levels as low stress (stress level 1 and 2), moderate stress (stress level 3) and high stress (stress level 4 and 5). Y-axis shows the prevalence of symptoms in percentage in each stress group. n: the number of students in each stress group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. **C.** Correlations Between Stress Level and Autonomic-Type Symptoms. Note. r: Pearson correlation coefficient. *$p < 0.05$; ***$p < 0.001$. This table shows the correlation between self-reported stress level and individual autonomic-type symptoms. All symptoms were significantly positively correlated with stress level ($p < 0.001$), with mood changes showing the strongest correlation ($r = 0.501$). **D.** Mean Symptom Count and Percentage of Students with ≥3 Symptoms Across Self-Reported Stress Levels. Note. SD: standard deviation. This table shows the relationship between self-reported

stress level and the number of autonomic-type symptoms experienced by students. Higher stress levels were associated with greater mean symptom values and a higher percentage of students who reported three or more symptoms.

The results showed significant positive correlations between stress and all 13 symptoms, with the strongest correlation was for mood changes ($r = 0.501$) (see Table 6). Also, it was noted that the symptom burden increased as the stress level increased. The students with a very high stress level reported the highest mean symptom count, and 3 or more symptoms were noted in 87% of these students (see Figure 3D).

5- Symptom count was found to be significantly lower in the regularly exercising group

The mean symptom count was found to be significantly lower in the regularly exercising group than the non-exercising group (Table 4) (4.00±2.81 vs. 4.94±3.21, respectively; p = 0.003). The students in the exercising group reported less symptoms but the difference was significant only for "shortness of breath after activity" (p = 0.015).

Table 4. Autonomic-Type Symptom Prevalence by Regular Exercise Status.

Symptom	Exercising Group (n=261)	Non-Exercising Group (n=140)	p-value
Lightheaded/dizzy after standing	60.5%	67.1%	0.183
Brain fog / difficulty focusing	44.8%	55.7%	0.055
Headaches	41.8%	47.9%	0.288
Mood changes (anxiety/depression)	38.7%	47.1%	0.091
Spots/blurred vision after standing	40.2%	46.4%	0.300
Cold hands or feet	31.0%	40.7%	0.069
Ringing in the ears	32.6%	40.7%	0.126
Feeling unusually overheated	21.8%	27.1%	0.318
Shortness of breath after activity*	17.2%	27.9%	0.015
Heart racing/pounding at rest	23.0%	31.4%	0.090
Chest pain or tightness	18.4%	25.7%	0.099
Fainting or feeling faint	12.3%	19.3%	0.071
Flushed/reddened skin	14.2%	20.0%	0.158

*Note. n: number of participants. * $p < 0.05$*

This table compares the prevalence of autonomic-type symptoms between participants who exercise regularly and those who do not. Shortness of breath after activity was the only symptom showing a statistically significant difference (p=0.015)

6- A high mean symptom count was recorded in the students sleeping less than 6 hours per night

The results showed significantly high mean symptom count in the students sleeping less than 6 hours per night compared to the ones sleeping 6 hours or more (Figure 4) (4.91±3.15 vs. 3.96±2.88, respectively, p = 0.005). Figure 4 shows the symptom prevalence by sleep duration

and it demonstrates a significantly high prevalence of all symptoms in students with less than 6 hours of daily sleep.

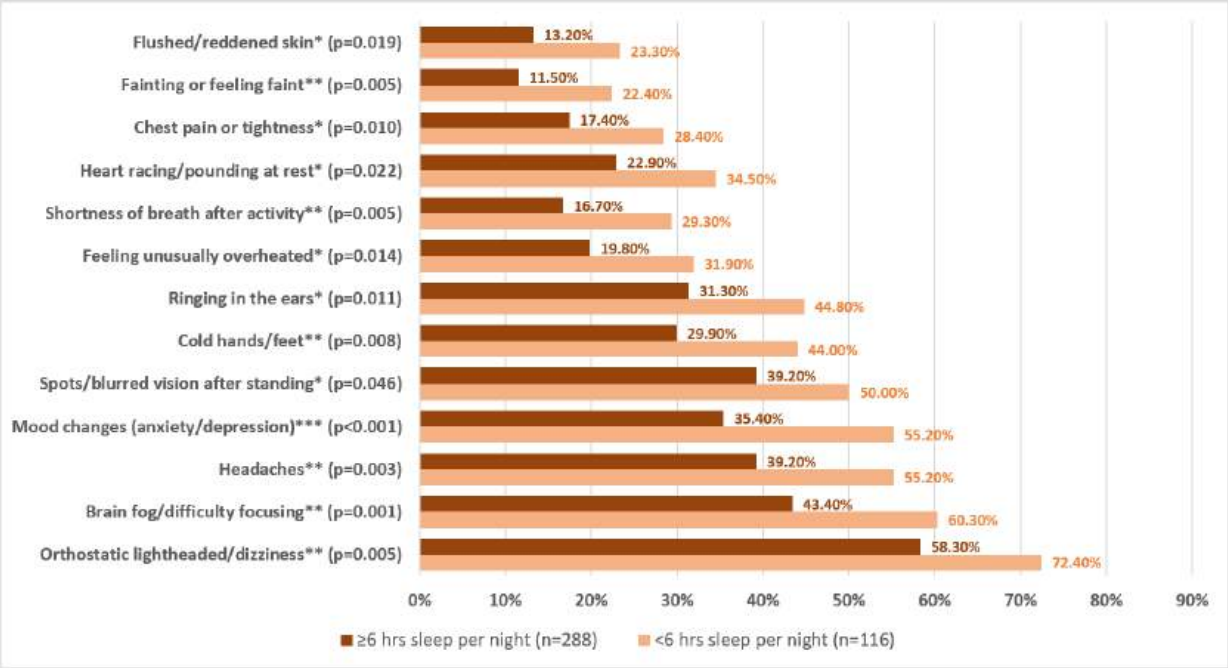


Figure 4. Autonomic-Type Symptom Prevalence by Sleep Duration. X-axis shows percentage of the symptoms by sleep duration, Y-axis shows symptoms with significance level (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) and statistical p values.

7- Mean symptom count across the water intake groups was significantly high in the dehydrated group

The symptom prevalence by water intake is shown in Table 5. The mean symptom count across the water intake groups was significantly high in the dehydrated group (who has daily water intake of 0-1 cup) compared to the groups with moderate and high amount of water intake (One-Way ANOVA $F = 2.95$, $p = 0.0327$), however only "shortness of breath after activity" showed statistical significance across all the water intake subgroups.

Table 5. Autonomic-Type Symptom Prevalence by Daily Water Intake.*This table compares the prevalence of autonomic-type symptoms across different levels of daily water intake. Most symptoms decreased as water intake increased, with shortness of breath after activity showing a statistically significant correlation with water intake (p = 0.047).*

Symptom	0–1 cups (n=33)	2–4 cups (n=131)	5–6 cups (n=146)	>6 cups (n=87)	p-value
Orthostatic lightheadedness/dizziness	72.7%	64.1%	61.0%	60.9%	0.408
Brain fog / difficulty focusing	60.6%	51.9%	46.6%	43.7%	0.180
Headaches	57.6%	46.6%	41.8%	40.2%	0.187
Mood changes (anxiety/depression)	54.5%	43.5%	39.0%	37.9%	0.141

Symptom	0–1 cups (n=33)	2–4 cups (n=131)	5–6 cups (n=146)	>6 cups (n=87)	p-value
Spots/blurred vision after standing	54.5%	44.3%	40.4%	40.2%	0.261
Cold hands/feet	48.5%	37.4%	31.5%	28.7%	0.117
Ringing in the ears	48.5%	37.4%	33.6%	32.2%	0.173
Feeling unusually overheated	30.3%	24.4%	22.6%	20.7%	0.378
Shortness of breath after activity*	36.4%	22.9%	19.2%	16.1%	0.047
Heart racing/pounding at rest	39.4%	28.2%	24.0%	20.7%	0.064
Chest pain or tightness	33.3%	21.4%	19.9%	17.2%	0.104
Fainting or feeling faint	24.2%	16.0%	14.4%	11.5%	0.183
Flushed/reddened skin	24.2%	17.6%	15.1%	13.8%	0.238

*Note. * $p < 0.05$. Mean symptom count: 5.76±3.55 in 0–1 cup group; 4.27±2.85 in 2–4 cups group; 4.06±2.99 in 5–6 cups group; and 4.32±2.90 in >6 cups group (One-Way ANOVA $F = 2.95$, $p = 0.0327$).*

8- Pearson correlations between groups

Figure 5 shows Pearson correlations between groups. Female gender, joint hypermobility, regular exercise, and more than 6 hours of daily sleep were also significantly correlated with the total symptom count. Significant correlation was found to be between stress and all 13 symptoms with the strongest correlation being for mood changes ($r = 0.501$) (see Figure 5A). Figure 5B is the correlation between each predictor and total symptom count. Perceived stress was to have the strongest correlation ($r = 0.413$, $p < 0.001$). Symptom burden increased as the stress level increased. The students with a very high stress level reported the highest mean symptom count, and 3 or more symptoms were noted in 87% of these students (Figure 5C).

Moreover, a two-way ANOVA test was used to analyze how gender and joint hypermobility status interact on mean symptom count. The results showed that the main effects of both gender and joint hypermobility were significant, females reported higher symptom counts ($p<0.001$), and students with joint hypermobility in themselves or in their families did so as well ($p=0.011$). However, the interaction between gender and hypermobility did not reach statistical significance ($p= 0.072$) in this sample.

A **Correlations Between Predictors and Total Symptom Count.**

Predictor	Pearson r	p-value
Stress level***	0.413	<0.001
Being female***	0.298	<0.001
Joint hypermobility**	0.157	0.001
Regular exercise**	−0.149	0.006
Hours of sleep**	−0.131	0.009
Water intake	−0.082	0.101 (NS)
Grade level	−0.000	0.996 (NS)

B **Correlations Between Stress Level and Autonomic-Type Symptoms.**

Symptom	r (with stress)	r^2 (% variance)	p-value
Mood changes (anxiety/depression)	0.501	25.1%	<0.001***
Headaches	0.368	13.5%	<0.001***
Heart racing/pounding at rest	0.355	12.6%	<0.001***
Brain fog/difficulty focusing	0.301	9.1%	<0.001***
Fainting or feeling faint	0.296	8.8%	<0.001***
Shortness of breath after activity	0.280	7.8%	<0.001***
Flushed/reddened skin	0.275	7.6%	<0.001***
Chest pain or tightness	0.271	7.3%	<0.001***
Orthostatic lightheadedness/dizziness	0.260	6.8%	<0.001***
Spots/blurred vision after standing	0.239	5.7%	<0.001***
Feeling unusually overheated	0.237	5.6%	<0.001***
Ringing in the ears	0.191	3.6%	<0.001***
Cold hands/feet	0.126	1.6%	0.017*

C **Mean Symptom Count and Percentage of Students Reporting ≥3 Autonomic-Type Symptoms Across Stress Levels.**

Stress Level	n	Mean Symptom Counts±SD	% with ≥3 Symptoms
1 — Very Low	27	2.19±2.06	33.3%
2 — Low	66	2.83±2.53	43.9%
3 — Moderate	153	4.10±2.62	71.2%
4 — High	124	5.13±2.80	83.9%
5 — Very High	**23**	**7.83±3.60**	**87.0%**

Figure 5. Pearson correlations between groups A. This table shows a significant positive correlation for stress level and female gender (increase in stress is associated with increased total symptom count; female students report higher number of symptoms). It also shows positive correlation between joint hypermobility and symptom count, and negative correlation between sleep duration and symptom count. **B.** This table shows the strength of the relationship between self-reported stress level and individual autonomic-type symptoms, including the proportion of variance resulting from stress. Mood changes showed the strongest correlation with stress ($r = 0.501$; 25.1% variance), while all listed symptoms were significantly correlated with stress ($p < 0.05$).*$p < 0.05$; ***$p < 0.001$. B. Note. ** $p < 0.01$; *** $p < 0.001$. NS: not significant. **C.** This table shows how the number of autonomic-type symptoms vary across self-reported stress levels. As stress increased, both the mean symptom value and the percentage of students experiencing three or more symptoms increased. SD: standard deviation.

9-Risk Stratification and Accumulated Risk Factors

Table 6 and Figure 5 show the mean symptom counts by the number of concurrent 5 risk factors, including high stress level, female gender, joint hypermobility, not doing regular exercise, and sleep duration <6 hours per night. Students who carried all of the 5 risk factors reported a mean of 7.12±4.39 autonomic-type symptoms while this was 2.34±1.70 for the students without any risk factors.

Table 6. Risk Stratification based on Mean Symptom Counts and Numbers of Simultaneous Risk Factors.

# Risk Factors	n	Mean Symptom Counts±SD	Risk Category
0	41	2.34±1.70	Very Low
1	97	3.16±2.46	Low
2	139	4.45±3.07	Moderate
3	83	5.06±2.75	High
4	**36**	**6.69±2.67**	**Very High**
5	**8**	**7.12±4.39**	**Highest**

Note. Risk factors: High stress (≥4 stress level), female gender, joint hypermobility, no regular exercise, and <6 hours of sleep per night. This checklist identifies symptom burden, not diagnosis.

This table groups students by the number of simultaneous risk factors and their corresponding mean autonomic-type symptom burden. Mean symptom values increased as the number of risk factors increased, with students having four or five risk factors being classified in the very high or highest risk categories.

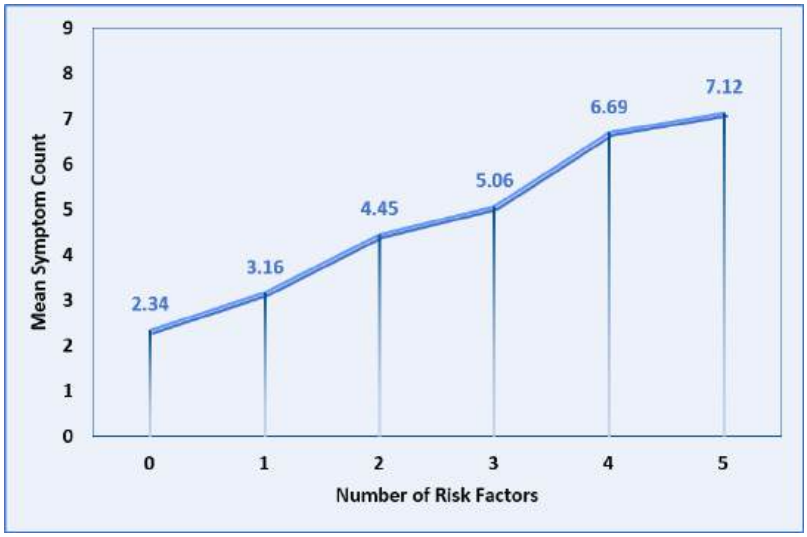


Figure 6: Mean symptom count by number of simultaneous risk factors. This figure shows the relationship between the number of simultaneous risk factors and mean autonomic-type symptom value. Mean symptom burden increased as the number of risk factors increased, rising from 2.34 symptoms with no risk factors to 7.12 symptoms with five risk factors.

10- Predicting Total Symptom Count

Multiple regression analysis was used to predict the total symptom count using seven predictors, and the findings are summarized in Table 7. Five of the seven predictors were found to be independently significant. Stress level was found to be the strongest predictor ($b = 1.040$, $p < 0.001$). Female gender, joint hypermobility and exercising regularly were also independently significant. Sleep duration and water intake were not independently significant when controlling for other factors. The multiple regression model explained 23% of total symptom variance ($R^2 = 0.230$), meaning 77% of variance is attributable to factors not captured in this survey (i.e.: genetics, prior diagnoses, medications, dietary patterns).

Table 7. Predicting Total Symptom Count.

Predictor	b	SE	p-value
Stress level* **	1.040	0.144	<0.001
Female gender* **	1.297	0.310	<0.001
Joint hypermobility*	0.668	0.297	0.025
Regular exercise*	−0.688	0.298	0.021
Grade level*	−0.338	0.163	0.039
Sleep duration	−0.158	0.108	0.145
Water intake	0.153	0.079	0.053

*Note. b = unstandardized regression coefficient. SE = standard error. * p < 0.05; *** p < 0.001 This table shows the regression results identifying factors associated with total autonomic-type symptom count. Higher stress level, female gender, and joint hypermobility were associated with greater symptom counts, while regular exercise and higher grade level were associated with lower symptom counts.*

11- Only 5.9% of the students reported having knowledge of dysautonomia

Table 8 summarizes the students' awareness of dysautonomia and the interest in learning more about this condition. The results showed that only 5.9% of the students reported having knowledge of dysautonomia, 94.1% of the students were noted to have inadequate awareness of this condition and 78.2% of the students were unaware of physical counter-maneuvers that help to reduce the orthostatic symptoms. Approximately 59% of the students reported to have interest in learning more about dysautonomia.

Table 8. Student Awareness of Dysautonomia.

Awareness Item	n	%
Never heard of dysautonomia	282	69.8%
Heard of it but do not understand it	98	24.3%
Heard of it and understand it	24	5.9%
Total with INADEQUATE knowledge	**380**	**94.1%**
Unaware of physical counter-maneuvers	316	78.2%
Interested in learning more	237	58.7%

Note. Counter-maneuvers = evidence-based physical techniques (e.g., leg-crossing, isometric muscle tensing, squatting, salt/fluid loading) that help to reduce the orthostatic symptoms. This table summarizes the students' awareness and understanding of dysautonomia and related symptom-management strategies. Most students (94.1%) had inadequate knowledge of dysautonomia, while 58.7% expressed interest in learning more about the condition.

DISCUSSION

This is the first study to systematically examine the symptoms that are commonly associated with dysautonomia (autonomic dysfunction) and to assess the student awareness of the condition in an American public high school population. Three principal findings were noted: First, autonomic-type symptoms were highly prevalent, with nearly two-thirds of students reporting orthostatic lightheadedness/dizziness and almost half of the students reporting brain fog. Second, perceived stress showed the strongest association with symptom burden, in both correlation analyses and multiple regression models. Third, awareness of dysautonomia was very limited despite the high frequency of reported autonomic-type symptoms.

In this research, the most commonly reported autonomic-type symptom was orthostatic lightheadedness/dizziness which is the hallmark feature of orthostatic intolerance. [8] Female students reported significantly greater symptom burden compared to male students, consistent with the known female predominance of POTS and related autonomic disorders.[10,11] At the same time, higher rates of anxiety and mood disorders among adolescent females may also contribute to this difference and should be considered when interpreting these findings.

Students who regularly exercise reported significantly less mean symptom count. This finding is consistent with the cardiovascular and autonomic benefits of regular aerobic exercise as deconditioning contributes to orthostatic intolerance.[16] Students reporting joint hypermobility also experienced greater symptom burden, consistent with previous reports linking connective tissue disorders and autonomic dysfunction.[11,12] In addition, symptom burden increased steadily as multiple risk factors accumulated. Students with four or five concurrent risk factors including high perceived stress, female gender, joint hypermobility, limited exercise, and short sleep duration reported the highest symptom counts. Although this pattern does not establish causation, it suggests that these identifiable characteristics may help school health professionals recognize students who could benefit from further evaluation.

The limited awareness of dysautonomia among students was one of the most important findings of this study. More than 94% of respondents reported little or no knowledge of dysautonomia, and over three-quarters were unfamiliar with physical counter-maneuvers that may help reduce orthostatic symptoms. At the same time, nearly 60% expressed interest in learning more about the condition. These findings suggest that school-based education about dysautonomia, healthy hydration, sleep habits, and when to seek medical evaluation could improve student awareness and encourage appropriate referral. Such education may benefit students regardless of whether their symptoms are ultimately caused by dysautonomia, anxiety, dehydration, anemia, or another medical condition.[17]

LIMITATIONS

Limitations in interpretation of the results

An important limitation of these findings is that prevalence of autonomic-type symptoms should not be interpreted as disease prevalence. The symptoms evaluated in this survey are commonly seen in patients with dysautonomia but nonspecific and may result from some other conditions that are common in adolescents such as anxiety, iron-deficiency anemia, dehydration, migraine, hypothyroidism, poor sleep, and physical deconditioning. Consequently, this study cannot determine how many students have dysautonomia or any other medical condition.

The strong relationship between perceived stress and symptom burden can be interpreted in more than one way. Stress may worsen symptoms in students with underlying autonomic dysfunction, as suggested by previous studies of POTS.[16] Alternatively, stress may independently contribute to many of the reported symptoms through anxiety, sleep disruption, behavioral changes, and increased sympathetic activation without primary autonomic dysfunction. The present study cannot distinguish between these possibilities.

Regardless of the underlying cause, students experiencing frequent dizziness, brain fog, palpitations, or fatigue may experience meaningful impairment in daily life. Therefore, these findings support the importance of appropriate medical evaluation rather than assuming that dysautonomia is the underlying cause.

Other limitations

This study has several other limitations: First, this study was conducted as a large pilot study at a single high school and may not generalize to the entire school and other student populations. Second, all data were self-reported and were not confirmed by clinical evaluation. Third, the symptom checklist was developed from published dysautonomia literature but was not a validated diagnostic instrument. Fourth, participation was voluntary, and recruitment through HOSA likely introduced selection bias, particularly toward students interested in health sciences and female students. Fifth, the cross-sectional design does not permit conclusions about causality. Sixth, joint hypermobility was assessed by self-report rather than a validated clinical measure such as the Beighton Score. Finally, multiple statistical comparisons were performed without formal correction because this study was exploratory, and the regression model explained only 23% of the observed variation in symptom burden, indicating that additional unmeasured factors likely contribute to symptom variability.

Overall, this study found that symptoms commonly associated with dysautonomia are frequently reported among high school students, while awareness of the condition remains very limited. These findings support the need for additional research in more diverse student populations and suggest that increasing awareness, encouraging healthy lifestyle habits, and promoting appropriate medical evaluation may help identify students who would benefit from further assessment.

CONCLUSIONS

This study found that self-reported symptoms commonly associated with dysautonomia are frequently reported among students at Seven Lakes High School. Nearly two-thirds of respondents experienced lightheadedness after standing, almost half reported brain fog, and more than two-thirds reported three or more symptoms. Perceived stress showed the strongest association with symptom burden, while female sex and joint hypermobility were also associated with higher symptom counts. Regular exercise was associated with lower symptom burden.

These findings should not be interpreted as evidence that dysautonomia itself is common among high school students. The symptoms evaluated are nonspecific and may result from many conditions prevalent during adolescence, including anxiety, sleep deprivation, dehydration, anemia, and other medical disorders. Nevertheless, the high frequency of reported symptoms, together with the very low level of awareness about dysautonomia and symptom-management strategies, suggests that many students experiencing these symptoms may benefit from greater health education and appropriate medical evaluation rather than assuming the symptoms are simply part of everyday academic life.

Future studies should include students from multiple schools and geographic regions, use clinically validated screening and diagnostic methods, and evaluate whether educational programs or early referral strategies improve symptom recognition and student well-being. Collaboration with pediatric cardiologists or autonomic specialists to clinically evaluate a subset of students reporting high symptom burdens would further strengthen understanding of autonomic dysfunction in adolescent populations.

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At the Root of the Problem: How the Oral Microbiome Links Type II Diabetes and Periodontal Disease By Olivia Shen

Abstract

This paper explores the relationships between periodontal disease, type II diabetes mellitus, and the oral microbiome. Periodontal disease is characterized by chronic inflammation and infection of the gingival tissues, while the hallmarks of type II diabetes mellitus are hyperglycemia and insulin resistance. The oral microbiome, the complex microbial community residing in the oral cavity, plays a significant role in the progression of both conditions. This paper also compares the oral microbiome and the gut microbiome to highlight their distinct characteristics and emphasizes the unique influence that the oral microbiome has on both type II diabetes and periodontal disease. This paper also conducts a meta-analysis that examines clinical studies published in endocrinology, periodontology, and biomedical peer-reviewed journals published between 2015 to 2025.

My analysis suggests that due to the limitations of current therapies, further research into oral microbiome-targeted treatments for periodontal disease and type II diabetes is necessary. This paper also reviews current treatments for these two diseases and ultimately argues that the oral microbiome is a currently underutilized avenue for innovative and cost-effective therapies. Given the growing global prevalence of type II diabetes mellitus, with approximately 1.2 million new diagnoses annually in the United States and 700 million diagnoses worldwide projected by 2040, improved treatment strategies are needed urgently.

Introduction

There are over 700 bacteria species living in your mouth. These species play diverse roles in oral health and mediating critical metabolic functions throughout the body. One of these roles includes protecting and supporting your immune system. Acid contained in vomit, for example, is extremely strong and could damage unprotected parts of the body. The bacteria in salivary glands in the mouth will produce extra saliva, or hypersalivation, before vomiting to protect the mouth. Additionally, they maintain oral health and dental hygiene. Oral bacteria species also contribute to decay and periodontal disease. These diseases begin to occur when there is dysbiosis, or harmful imbalance, in the oral microbiome.

The history of cavities extends almost as far back as human history. Cavities were first seen during the Neolithic Period, around 6000 years ago. This is the time when humans transitioned from hunting and gathering food, such as nuts, berries, grains, and meat, to farming. Farming produced a significantly higher amount of carbohydrates and grains. Consuming more carbohydrates can create dysbiosis in the mouth, which is a likely reason why cavities began to appear during this time. Changes to the oral microbiome and rates of dysbiosis have historically been linked to shifts in agricultural practices and the human diet.

Although there are many different microbiome communities throughout the body, the oral microbiome plays a critical role in the development of two physiological outcomes, namely type

II diabetes mellitus (T2DM) and periodontal disease. In this paper, I explore the role that diet plays in shaping the oral microbiome, emphasizing the role that carbohydrates play in periodontal disease and T2DM. Other critical factors that influence periodontal disease are dental hygiene and the ability to access appropriate preventive dental care. With this diversity of factors in mind, studies published from 2015-2025 are examined. This paper argues that the oral microbiome is a key mediator in the relationship between periodontal disease and type II diabetes mellitus. To do so, this paper evaluates the relationship between the oral microbiome in patients with type 2 diabetes mellitus, patients with periodontal disease, patients with both diseases, and patients with neither. The first section of the paper elaborates on the link between the oral microbiome and the progression of periodontal disease and type 2 diabetes. The second section of this paper compares the oral microbiome to the gut microbiome and argues that more research should be devoted to the oral microbiome. The final section of this paper explores current and potential treatment options for periodontal disease and type 2 diabetes that influence the oral microbiome. This paper concludes by suggesting multiple directions for future research.

1. **Literature Review**

There are many variables that affect the composition of the oral microbiome. According to Rajasekaran et al., factors such as smoking, alcohol consumption, and diet affect the microbiome composition. Smoking can affect salivary pH, produce an altered immune response, and facilitate anaerobic conditions that lead to the proliferation of pathogenic oral microorganisms. Nicotine, contained in cigarettes, can increase the amount of detrimental bacteria by increasing the virulence of harmful microorganisms. This disrupts the equilibrium between harmful and beneficial bacteria, resulting in overgrowth of the harmful bacteria.

Similarly, the ethanol from alcoholic beverages can influence oral health. Alcohol consumption decreases flow rate of saliva and changes the composition of saliva, such as decreasing the amount of protein, and contributes to dysbiosis. Consumption of alcohol also causes impaired neutrophil function and decreases inflammatory cytokine production, both of which lead to harmful bacterial overgrowth and increased bone resorption. Alcohol use also alters oral microbiota by decreased *Lactobacillales* and increased *Streptococcus*.

Another factor that affects the oral microbiome is diet. Consuming excess carbohydrates encourages acid-producing bacteria to grow, such as *Streptococcus mutans*. They degrade the enamel on teeth and allow cavities to develop. The antioxidants found in fruits and vegetables can also influence the balance of bacteria in the mouth by reducing inflammation in the mouth and neutralizing acids. Altogether, many lifestyle factors greatly affect the composition of the oral microbiome, suggesting that oral treatment options are possible through daily habits.

In a meta-analysis conducted by Stöhr et al., researchers discovered that individuals who have periodontal disease have a greater risk of developing type II diabetes, and those diagnosed with type II diabetes will more likely have periodontal disease. These findings highlight that periodontal disease and type II diabetes demonstrate bidirectional association, meaning that those with one of these conditions are more likely to develop the other.

Type II Diabetes Mellitus is a metabolic disorder where the body resists insulin. It is characterized by high levels of glucose in the blood. When insulin resistance occurs, beta cells in the body produce extra insulin, and over time, the pancreas slows production due to prolonged overproduction. Unlike type I diabetes, which is usually genetic and is considered more of an autoimmune process, type II diabetes is caused by lifestyle choices, including diet, with some contributions from genetics. Additionally, type 1 diabetes is often diagnosed in children, whereas type 2 diabetes generally first presents in adulthood. Around 1.2 million Americans are diagnosed with diabetes every year, and it is predicted that by 2040, 700 million people will be diagnosed with diabetes globally.

The term periodontal disease refers to an infection and inflammation of the gums and structures supporting the teeth, such as the gingiva and the mandible. It could be caused when plaque, a form of biofilm, forms on the teeth and hardens into tartar. When bacteria is trapped under the tartar, the gums experience irritation and can produce systemic inflammation. The infection can also spread to surrounding bones. Even though periodontal disease can be reversible in the early stages, the symptoms are not easily noticeable, therefore delaying treatment and approaching the irreversible damage of the later stages.

Another factor that could lead to periodontal disease is dysbiosis. Dysbiosis is an imbalance in the composition, function, or diversity of the microorganisms in the oral microbiome. Under healthy conditions, the bacteria positively contribute to the body by helping to balance the mouth's pH and breaking down carbohydrates. When the bacteria's balance is disrupted, dysbiosis occurs, sometimes due to high intake of carbohydrates and smoking. This can lead to an increase in harmful bacteria, such as *Streptococcus mutans,* a decrease in diversity, dental caries, and periodontal disease. In addition to these environmental influences, there are also non-environmental factors that play a role in shaping the oral microbiome. Genetics, in particular, can significantly affect the composition of the oral microbiome. A study conducted by Springer Nature discovered that identical twins, regardless of gender, had more similar oral microbiome compositions than fraternal twins or unrelated individuals. They also found that there are two genetic regions that highly correlate with the most heritable oral microbiome traits, on chromosome 7, located near the gene *IMMPL2,* and on chromosome 12, located near the non-coding RNA gene *NHBA-AS1*. Additionally, individuals living together have a similar oral microbiome composition than those who do not live together.

2. Oral Microbiome and the Progression of Diabetes and Periodontal Disease

One of the most diverse bacterial ecosystems in the body is present in the oral cavity, commonly referred to as the oral microbiome. It includes hundreds of bacterial species, including *streptococcus mutans*. Within the oral cavity, there are many different habitats in which the oral microbiota reside, including, but not limited to, the teeth, gingival sulcus, tongue, cheek, hard and soft palates, and tonsils. Although there are around 700 species present, only 280 species have been named. When the mouth is dysbiotic, the oral microorganisms can cause a diverse

number of oral infections and diseases, including dental caries, periodontitis, endodontic infections, and tonsillitis.

Similar to the oral microbiome, the gut microbiome includes bacteria, virus, fungi, and archaean organisms. The gut microbiota are present in the gastrointestinal tract and colon. These microorganisms play a large role in the body's digestion, especially in the conversion of carbohydrates and glycans to short-chain fatty acids that provide energy, metabolism, and protection against pathogens. While both gut and oral microbiome communities both contribute to health and strengthen the immune system, they differ in local physiologic conditions. The gut microbiome exists in a mainly anaerobic, nutrient-rich environment, while the oral microbiome composition is exposed to oxygen and is influenced by a variety of external factors, such as dental hygiene, and food intake. Furthermore, the bacteria inhabiting the gut and oral ecosystems are significantly different, the mouth hosts bacteria that thrives in a pH neutral environment, and when the oral microbiota are swallowed, the high acidity of the stomach inhibits most of them. As a result, only a small number of these microbes reach the intestines. The acidity of the stomach acts as a barrier between the oral and gut microbiomes, further emphasizing the distinction between the two bacterial communities. Diseases that affect the oral microbiome could also possibly affect the gut microbiome, such as *P. gingivalis*.

The oral microbiome plays a large role in overall health and can reflect the condition of the body. For this reason, a healthy oral microbiome is extremely important. Usually in a healthy microbiota community, fungi and archaea are present. Moreover, on a more tangible scale, fresh breath, clean teeth, and healthy gums can indicate a healthy oral microbiome, whereas tooth decay can be a possible sign of poor oral microbial health. An unhealthy oral microbiome is usually caused by dysbiosis, which can lead to different oral diseases, such as periodontal disease, and possibly other illnesses within the body, including type 2 diabetes.

As mentioned previously, periodontal disease and type 2 diabetes mellitus are bidirectional. Type 2 diabetes, a chronic hyperglycemia disease, contributes to increased inflammatory response and slower wound healing. These changes could possibly lead to a higher susceptibility to developing periodontal infection and destruction of gingival tissue. Conversely, in periodontal disease, a chronic inflammatory condition, molecules are released that amplify inflammation, which contribute to insulin resistance. Diabetes and periodontal disease can exhibit comorbidity, and often do given the similar lifestyle factors that facilitate each disease. On the other hand, it has also been shown that periodontal disease treatment has led to an improvement in glycemic control.

When oral dysbiosis occurs, harmful bacteria usually tend to increase, causing inflammation that could enter the bloodstream. This can lead to cytokines interfering with insulin signals and lead to insulin resistance. Furthermore, bacteria can enter blood circulation and increase insulin resistance. In diabetes, healing is slower and immune cells respond less effectively, making periodontal disease and gum infection harder to treat.

3. Comparing and Contrasting the Oral Microbiome and Gut Microbiome

To understand the link between the oral microbiome and its effect on type 2 diabetes and periodontal disease, it is helpful to compare it with another microbiome, the gut microbiome. The oral microbiome and the gut microbiome differ significantly in their function, environment, and microbial composition. The oral microbiome inhabits the oral cavity, in which they are exposed to saliva, food, and oxygen. As a result, the microorganisms reside on hard and soft tissues, such as teeth and mucosa, as a biofilm, one form of which is dental plaque. In contrast, the gut microbiome is in an anaerobic environment in the colon. These gut microorganisms help with digestion and strengthen the immune system.

Although both microbiome communities play a significant role in human health, there has been more research on the gut microbiome. A reason for the disparity in research attention towards the gut microbiome is because it is larger than the oral microbiome, with more microbial species. Greater surface area might suggest greater importance, although this thinking is oftentimes incorrect, prompting researchers to direct their attention to the gut microbiome. Additionally, the microbiome in the gut is easier to access the bacteria to study though stool. Oral microbiome sampling, on the other hand, is more difficult due to an increased amount of microenvironments and many distinct niches, including tongue, teeth, hard palate, gums, saliva, and plaque.

Biofilms are communities of bacterial cells in a self-produced polymeric matrix. These biofilms are characterized by the specific microorganisms that reside in that particular organ and within that matrix. The bacteria and fungi attach to each other and a surface, such as teeth or tongue. Oral biofilms serve as a protective barrier for the mouth. There are biofilms that positively impact health and biofilms that are harmful. Beneficial biofilms preserve the healthy balance of the oral microbiome and prevent bad bacteria from developing. Harmful biofilms, including plaque, can lead to periodontal disease and dental caries. They form when there is a decrease in dental hygiene and an excess of sugary or acidic foods are consumed.

Dysbiosis, which is a disruption in normal function or composition of bacterial communities, can be found in both the oral and gut microbiomes, and it can lead to diseases, such as diabetes, in both. In the oral cavity, dysbiosis results in the loss of bacterial diversity, as well as an imbalance of bacteria where the concentration of pathogenic bacteria increase and the beneficial bacteria decrease. When diversity of bacteria in the mouth diminishes, the interactions between different species are disrupted. These interactions include mechanisms and antagonistic interactions that prevent harmful bacteria overgrowth. Consequently, microbial homeostasis is undermined which increases the susceptibility of the oral microbiome to dysbiosis. Additionally, it has been linked to various diseases, such as diabetes, inflammatory bowel disease, neurodegenerative disorder, and cardiovascular disease. There are many factors that could likely contribute to dysbiosis in the mouth. Some examples are poor oral hygiene, consuming excessive sugar and processed foods, medications and antibiotics, systemic factors, such as stress and weakened immune system, systemic diseases, smoking and alcohol. Symptoms and results of oral dysbiosis include periodontitis, halitosis, dental caries, gingivitis, dry mouth, and canker sores.

In contrast, gut dysbiosis is characterized by the imbalance of bacteria characterized by overgrowth of pathogenic bacteria and decreased diversity of microbiota in the gastrointestinal tract. There are both intrinsic factors, which harms the microbiome mildly, and extrinsic factors, which might cause irreversible damage to the gut, that contribute to gut dysbiosis. Intrinsic factors that can lead to dysbiosis include genetics, age, and intestinal diseases; extrinsic factors include the geographical location, lifestyle, and exposure to xenobiotics (chemicals that are not naturally produced by host organisms), can all alter the composition of the gut microbiome. Antibiotics have also been found to decrease microbial diversity and contribute to gut dysbiosis. Similar to the oral microbiome, dysbiosis in the gut microbiome can also lead to diseases, such as inflammatory bowel disease, malnutrition, diabetes, liver cirrhosis, and neurologic disorders.

Across multiple meta-analyses, there are more publications on the gut microbiome. Research from PubMed reveals that there are approximately 746 oral microbiome articles compared to 5605 gut microbiome articles, making there 7.5 times more articles on the gut microbiome than the oral microbiome. More research should be done on the oral microbiome to discover its therapeutic potential in treating periodontal disease and type 2 diabetes.

4. Potential Treatment Options for periodontal disease and type 2 diabetes

There are many dental treatments currently utilized. Some of the traditional treatment methods include drilling and filling cavities, which involves removing decayed tooth material and filling the space with resin or amalgam. There are also more modern techniques to treat dental caries. One of these approaches includes radiographic assessment. The radiographic examinations can detect caries between the teeth, assess bone levels for periodontal disease, and identify abscesses and tumors. One treatment for periodontal disease is drug delivery systems, or DDSs. DDSs are special materials and technologies based on nanotechnology that were developed by researchers to release drugs more effectively to the affected gums. They were originally created to control infection but are currently used to regenerate bone and tissue.

In addition to periodontal disease and dental caries treatment methods, there are also several drugs to treat type 2 diabetes. Glucagon-like peptide, also known as GLP-1, is a current treatment for type 2 diabetes. The hormone GLP-1 in the body is released by the gut after eating. It increases insulin release and helps maintain sugar levels through the incretin effect. However, natural GLP-1 is broken down quickly by the enzyme DPP-4. The drug GLP-1 receptor agonists stimulate the GLP-1 receptors and lower blood sugar. They act like the natural GLP-1 but last for a significantly longer amount of time. There are also DPP-4 inhibitors that block the natural DPP-4 enzyme to allow the GLP-1 receptors to last longer.

Although GLP-1 is an effective treatment for type 2 diabetes, there are also several drawbacks. For many, this drug is expensive and difficult to afford. Also, there are negative side effects as a result of the GLP-1 treatment, such as nausea and vomiting, mood changes and anxiety/depression, and possibly the development of Thyroid C-cell cancer and pancreatitis. Its long term effects are also unclear. Since treatments for type 2 diabetes usually require lifelong

management, the absence of long-term data presents the concern regarding the potential detrimental effects in the future, highlighting the need for continuous research.

Other options for type 2 diabetes treatment include metformin (Glucophage). It is a drug that lowers glucose levels. Like GLP-1 and many other treatments, metformin also has potential adverse effects. Up to 50% of patients reported nausea, diarrhea, and gastrointestinal cramping as a result of taking metformin, and an estimated 5% of patients experience intolerance to the medication. Additionally, though rare, high levels of biguanide drugs like metformin can impair mitochondrial function, leading to increased lactate production and, in severe cases, lactic acidosis. Lactic acidosis is a severe and potentially fatal condition characterized by accumulation of lactic acid in the blood. It usually occurs when cells rely on anaerobic metabolism, which results in excessive lactate production.

As demonstrated above, current treatments for periodontal disease and type 2 diabetes can often pose detrimental effects to patients. Conventional approaches can often be costly and potentially risky. Developing treatments that target the oral microbiome for these diseases may offer a more effective alternative. Compared to existing therapies, oral microbiome treatments can prove to be more cost effective, more comfortable, and safer to treat periodontal disease and mitigate insulin resistance.

5. Conclusion - Direction for future research

Both diabetes and periodontal disease are extremely prevalent yet preventable diseases in today's world. There could be a large variety of new and innovative treatment methods that target the oral microbiome that would ultimately treat both periodontal disease and type 2 diabetes mellitus to the interconnected, bidirectional nature of these two physiological conditions. Since diabetes is a systemic disease, accessing the oral microbiome would be easier than manipulating the endocrine system or the bacterial roots of dental infections. One promising approach involves the use of prebiotic and probiotic supplements to help create a more balanced oral microbial environment. These beneficial microorganisms have been shown to lower blood sugar, improve glycemic control, decrease insulin resistance, and decrease inflammation, which are all factors in the progression of both periodontal disease and type 2 diabetes. Because of these benefits, the therapeutic potential of probiotics and prebiotics should be further explored. In addition to their benefits in the body, these treatments may offer less expensive and more accessible alternatives to existing treatments. Probiotics and prebiotics could be incorporated into everyday oral hygiene products, such as mouthwashes and toothpastes. For example, chlorhexidine mouthwashes are common antiseptic tools in outpatient dental settings, suggesting that similar implementations of probiotics and prebiotics in oral hygiene products is feasible.

Another possible future research opportunity for treatment could be performing a study comparing individuals with type 2 diabetes, individuals with periodontal disease, as well as those with neither to analyze the oral microbial composition of those patients. Doing so could lead to identifying a common bacterial strand, such as *streptococcus,* that causes the diseases. After discovering the bacteria, researchers can possibly develop a probiotic or prebiotic chewing gum

or drink that targets that specific bacteria to see if that decreases the patient's hyperglycemia. This could be a more affordable and accessible treatment than options such as GLP-1.

Besides these possible treatments that could prove effective, the fundamentals should also be addressed. As discussed earlier in this paper, reducing excess sugar and carbohydrates from the diet, decreasing alcohol consumption, and maintaining dental hygiene are all key factors in supporting oral health and ultimately addressing the underlying causes of periodontal disease.

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NR3C1 Mediated Epigenetic Modifications Influencing the Nervous-System

By Agnes Olsson[1]

Abstract

Epigenetic mechanisms can influence gene expression without changing the underlying DNA sequence and may therefore connect daily experiences with nervous-system and hormonal regulation. This paper examines how stress-related experiences may affect hormonal regulation through epigenetic modifications, using the NR3C1 gene, specifically. NR3C1 encodes the glucocorticoid receptor, which is involved in cortisol signalling and regulation of the hypothalamic-pituitary-adrenal axis. This paper reviews associations between prenatal stress, early-life adversity, later trauma and changes in NR3C1 DNA methylation, as well as the possible effects of these changes on glucocorticoid-receptor expression and hormonal feedback.

In silico analyses were performed to investigate the properties of the NR3C1 protein. TMHMM is used to predict transmembrane regions, Clustal Omega is used to compare NR3C1 protein sequences from human, chimpanzee, pig and mouse, and STRING is used to examine known and predicted protein associations. A theoretical CRISPR/Cas9 gene-editing design is also included to generate mutations to establish a framework for future experimental research.

This paper highlights how environmental exposure, epigenetic regulation, nervous-system activity and hormonal signalling may interact at several biological levels. The findings support the importance of studying NR3C1 not only as a gene involved in cortisol signalling, but also as a protein whose function depends on its intracellular location, conserved structure and interactions with supporting proteins.

Keywords: nervous system, hormonal regulation, epigenetics, NR3C1, CRISPR/Cas9

1. Introduction

A hormone is a substance produced within an organism that acts as a chemical messenger. It helps regulate and coordinate functions in cells, tissues, and organs, contributing to processes such as metabolism, behaviour, and homeostasis (STÁRKA and DUŠKOVÁ 2020). Hormonal regulation is mediated by the nervous system because hormones can reach and influence the Central Nervous System (CNS). More specifically, hormones produced in the peripheral target glands, such as cortisol, estrogen, progesterone and testosterone, or in the anterior pituitary, including growth hormone (GH), thyroid stimulating hormone (TSH), prolactin (PRL) adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and melanin-stimulating hormone (MSH), are able to influence brain activity (Jack Westin 2020). The human hormonal system, also called endocrine system, is a network of glands that produce hormones to regulate body functions. These hormones influence processes such as growth and development, metabolism, electrolyte balance, and reproduction.

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The system includes the hypothalamus and pituitary gland, as well as other hormone-producing glands. Hormones released by the hypothalamus regulate the pituitary gland, and pituitary hormones, such as ACTH, gonadotropins, TSH, GH, PRL, oxytocin and vasopressin, can either act directly on target tissues or stimulate other glands to release additional hormones. In this way, hormones can operate through connected regulatory pathways within the body (Hiller-Sturmhöfel and Bartke 1998). This is possible because these hormones can cross the blood-brain barrier, enter the cerebrospinal fluid and penetrate brain tissue. Therefore, hormonal signals can participate directly in processes, such as learning, memory, sleep cycle, body movement, hormone regulation and many others (Reis et al. 2009) occurring within the CNS. The effects of hormones on the CNS can be classified into permanent and temporary effects. Permanent effects occur mainly during prenatal or early postnatal brain development and persist without the continued presence of the hormone. Temporary effects depend on the continued presence of the hormone and end after the hormone is metabolized or eliminated (Martini 1969).

Steroid hormones are specifically relevant to epigenetics because DNA methylation (the addition of methyl groups to DNA, it can affect whether a gene is expressed) and histone modifications (chemical changes to histone proteins, these changes can affect the regulation of gene expression) can regulate genes that encode key enzymes involved in steroid-hormone biosynthesis. Differential DNA methylation was identified near the transcriptional start sites of genes such as CYP11A1, HSD3B1 and CYP19A1. DNA methylation was also involved in permanently silencing CYP19A1 expression in lutein cells. Since CYP19A1 encodes the enzyme that converts androgens to estrogens, this regulation affects a key step in steroidogenesis. DNA methylation also regulated CYP17A1 expression, while histone modifications regulated expression of the STAR gene. Together, these findings show that epigenetic mechanisms can regulate genes involved in steroid-hormone biosynthesis. Glucocorticoids are relevant because epigenetic changes can affect glucocorticoid receptor expression. Histone H3K9 acetylation and reduced methylation in the glucocorticoid receptor promoter were associated with increased receptor expression in fetuses of undernourished ewe that developed obesity later in life. Thyroid hormones can be included because the sodium iodide supporter, SLC5A5, which is responsible for iodine uptake in the thyroid, is regulated by cytosine methylation of its promoter. This means that DNA methylation can influence expression of a gene involved in iodine uptake in the thyroid. Calcitriol and parathyroid hormone (PTH) provide a clear example of a hormone influencing an epigenetic process. DNA methylation regulates CYP27B1, which encodes the enzyme responsible for the final step in vitamin D bioactivation. Vitamin D represses CYP27B1 expression, while PTH stimulates it by inducing demethylation of the CYP27B1 promoter (Zhang and Ho 2011).

In complex organisms, steroid, retinoid and thyroid hormones regulate homeostasis and development, and their effects are mediated by intracellular receptor proteins that act directly on genetic regulation. In the nervous system, glucocorticoid hormones are connected to type I and type II corticosteroid receptors that are found in the nervous system, including mineralocorticoid and glucocorticoid receptors. Glucocorticoid hormones therefore fit into a broader receptor

system in which steroid hormones act after entering the cell. Because steroid hormones are lipophilic, they can pass through the cell membrane and bind to intracellular receptor proteins. Once the hormone binds, the receptor is changed so that it can recognize specific DNA sequences. These sequences are called hormone response elements and regulate the transcription of nearby target genes. In this way, the relationship between hormonal regulation and the nervous system is based on corticosteroid receptors in the nervous system and the ability of hormone-bound receptors to regulate gene expression (Evans and Arriza 1989).

Beyond direct receptor action in nervous system cells, steroid hormones may also act through glia cells. Glia participate in brain function throughout life and are sensitive to steroid hormones. Since glia cells have steroid receptors, and since promoter regions of glial-specific genes such as glutamine synthetase contain hormone-responsive elements, they may be direct targets for steroid hormones. Glia may also mediate steroid action on the nervous system through steroid metabolism, brain development, synaptic plasticity, neural repair and aging and brain immune function. Steroids may further affect communication between glia and neurons by altering glial production of secreted and cell-surface signaling molecules and/or neuronal sensitivity to those signals. (Jordan 1999)

The nervous system can be described as a central regulatory system made up of interconnected neurons and supporting glial cells. In the CNS, gray matter contains neuronal cell bodies, while white matter consists mainly of axons. Neurons receive and transmit signals through their dendrites, axons and synapses, allowing nerve cells to communicate with one another and form neural networks. The CNS consists of the brain and spinal cord, while the Peripheral Nervous System (PNS) consists of nervous structures outside the brain and spinal cord. The PNS connects the CNS with the rest of the body. (Brodal 2010)

Epigenetics refers to processes that influence gene expression via gene regulation mechanisms. These processes affect how genetic information is used and act mainly during transcription, although other stages, including translation, may also be regulated epigenetically. Examples of epigenetic processes include DNA methylation, histone modification and different forms of RNA-mediated regulation. The regulation of gene expression is important because cells within the same organism contain essentially the same DNA but can differ greatly in their functions and characteristics. These differences arise because genes are expressed in different ways and in different amounts in different cell types. Epigenetic processes contribute to this regulation by influencing gene expression. The control of gene expression is therefore central to differentiation and development, allowing cells with essentially the same DNA to develop different functions (Gibney and Nolan 2010).

A specific gene relevant to hormonal stress regulation is NR3C1, which encodes the glucocorticoid receptor. The glucocorticoid receptor is involved in several stages of modulation of the hypothalamic-pituary-adrenal axis (HPA). The HPA axis has been reported to be dysregulated both in individuals exposed to early psychosocial stress and in people with several psychiatric disorders. NR3C1 methylation has therefore been investigated in relation to early-life stress, stress related disorders, glucocorticoid-receptor expression and psychosocial stress

reactivity. NR3C1 has a complex regulatory structure. It contains eight coding exons, numbered 2-9, and nine alternative non-coding first exons. These first exons are considered to act as alternative promoters. Several of them are positioned within a CpG island, a DNA region containing many CpG sites (specific positions in a DNA sequence where cytosine is followed by guanine), in the proximal promoter region of a gene. This CpG island extends across approximately three kilobases and contains around 300 CpG sites that may be affected by methylation. Several forms of early life stress were frequently associated with increased NR3C1 methylation. These included prenatal stress, childhood maltreatment and early parental loss. In contrast, reduced NR3C1 methylation was repeatedly associated with post-traumatic stress disorder. Therefore, both increased and decreased methylation may be maladaptive, depending on factors such as timing and type of stress exposure.

NR3C1 methylation has also been associated with expression of the glucocorticoid receptor. Increased methylation in promoter regions following early adversity may reduce glucocorticoid receptor expression and contribute to glucocorticoid resistance. In contrast, demethylation associated with later acute trauma may increase receptor expression and contribute to greater sensitivity of the HPA axis (Palma-Gudiel et al. 2015).

Prenatal exposure to maternal depression was associated with epigenetic modification of NR3C1, the gene encoding the glucocorticoid receptor. Increased methylation was detected in exon 1F region of NR3C1 in newborn cord-blood samples when mothers experienced greater depressive symptoms during pregnancy. This association remained after prenatal treatment with serotonin reuptake inhibitors and postnatal maternal mood taken into account. The methylation change was also connected to later hormonal stress regulation. Greater NR3C1 methylation in newborns was associated with a larger salivary cortisol response to a stressor at three months of age. The findings therefore link a prenatal environmental condition with epigenetic modification of glucocorticoid receptor gene and altered cortisol reactivity during infancy. This represents a disruption in the regulation of the hormonal stress response rather than a mutation of NR3C1 or a diagnosed disease caused by the gene (Oberlander et al. 2008).

NR3C1 mutations have been identified in some cases of cushing disease, which is associated with ACTH-secreting pituitary tumors and excessive cortisol production. The identified mutations reduced normal glucocorticoid-receptor function, including movement of the receptor into the nucleus after glucocorticoid stimulation. They also weakened glucocorticoid-mediated suppression of POMC transcription, and one mutation increased ACTH secretion. These changes may contribute to impaired hormonal feedback in the pituitary tumor cells (Miao et al. 2021). Altered regulation of NR3C1 has also been linked to major depressive disorder following early-life stress. Early-life stress included experiences such as psychological trauma, child maltreatment, maternal separation and domestic violence. Across the studies examined, increased DNA methylation was observed in exon 1F of NR3C1 in relationship between early-life stress and major depressive disorder. Major depressive episodes were further connected to dysfunctional glucocorticoid-mediated negative feedback resulting from allostatic overload. These findings describe an association between environmental stress, epigenetic

alteration of NR3C1 and major depressive disorder rather than a mutation in the gene (Holmes et al. 2019).

In this study, the protein properties of NR3C1 aimed to be analysed and a CRISPR/Cas9 mediated gene editing designed to establish a framework that can be applied in experimental settings. Understanding the different mutant variations and their effect on gene regulation with NR3C1 will be essential to understand the differences in hormonal regulation.

2. Materials and Methods

Across the study several databases and in silico tools were used

NCBI, The National Center for Biotechnology Information (NCBI), is a collection of biological and medical databases. It provides information about genes, DNA and RNA sequences, proteins, genetic variants, scientific studies and different organisms. It also connects gene and protein records with related functions, diseases and research articles.

Ensembl, is a genome-browser database containing information about human and other organisms' genomes. It can be used to locate genes on chromosomes and examine exons, introns, coding regions, transcripts and genetic variants. It also provides DNA, RNA, and protein sequences.

TMHMM, is a computational tool that predicts transmembrane helices in protein sequences. These are hydrophobic regions that are likely to pass through a cell membrane. The program predicts their number and position and can indicate which parts of a protein are located inside or outside the membrane.

Clustal Omega, is a multiple-sequence-alignment tool used to compare DNA, RNA or protein sequences. It arranges corresponding nucleotides or amino acids together, making it possible to identify similarities, mutations, insertions, deletions, and conserved regions between sequences.

STRING Protein, is a database of known and predicted associations between proteins. It displays proteins as a network and shows possible physical interactions or functional relationships. The evidence may come from experiments, databases, scientific publications and computational predictions.

CRISPR Finder, also known as CRISPRCasFinder, is a bioinformatics tool used to detect CRISPR arrays and nearby cas genes in DNA sequences. It identifies repeated sequences, variable spacer regions, and possible CRISPR–Cas system types.

SnapGene, is molecular-biology software used to visualise, annotate, and design DNA sequences. It can display genes, coding regions, promoters, primers and restriction-enzyme sites. It can also simulate procedures such as PCR, restriction digestion, DNA assembly and molecular cloning.

3. Results

3.1. NR3C1 does not contain a transmembrane region

The NR3C1 protein sequence was analysed using TMHMM for testing whether the protein has any transmembrane region. The complete sequence of 777 amino acids sequence from NCBI>Protein was entered into the TMHMM algorithm. TMHMM checks the hydrophobicity of each amino acid and calculates the likelihood that it forms a transmembrane helix and that region is located inside a membrane, outside a membrane, or in a membrane-spanning region. The result showed that NR3C1 has zero predicted transmembrane helices (Figure 1). This means that NR3C1 is not a membrane protein. This result is important because NR3C1 is a receptor that works inside the cell. It binds glucocorticoid hormones and then helps regulate the activity of specific genes in the nucleus.

RecName: Full=Glucocorticoid receptor; Short=GR; AltName: Full=Nuclear receptor subfamily 3 group C member 1

UniProtKB/Swiss-Prot: P04150.1

GenPept Identical Proteins Graphics

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>sp|P04150.1|GCR_HUMAN RecName: Full=Glucocorticoid receptor; Short=GR; AltName:
Full=Nuclear receptor subfamily 3 group C member 1
MDSKESLTPGREENPSSVLAQERGDVMDFYKTLRGGATVKVSASSPSLAVASQSDSKQRRLLVDFPKGSV
SNAQQPDLSKAVSLSMGLYMGETETKVMGNDLGFPQQGQISLSSGETDLKLLEESIANLNRSTSVPENPK
SSASTAVSAAPTEKEFPKTHSDVSSEQQHLKGQTGTNGGNVKLYTTDQSTFDILQDLEFSSGSPGKETNE
SPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNEDCKPLILPDTKPKIKDNGDLVLSSPSNVTLPQVKTEK
EDFIELCTPGVIKQEKLGTVYCQASFPGANIIGNKMSAISVHGVSTSGGQMYHYDMNTASLSQQQDQKPI
FNVIPPIPVGSENWNRCQGSGDDNLTSLGTLNFPGRTVFSNGYSSPSMRPDVSSPPSSSSTATTGPPPKL
CLVCSDEASGCHYGVLTCGSCKVFFKRAVEGQHNYLCAGRNDCIIDKIRRKNCPACRYRKCLQAGMNLEA
RKTKKKIKGIQQATTGVSQETSENPGNKTIVPATLPQLTPTLVSLLEVIEPEVLYAGYDSSVPDSTWRIM
TTLNMLGGRQVIAAVKWAKAIPGFRNLHLDDQMTLLQYSWMFLMAFALGWRSYRQSSANLLCFAPDLIIN
EQRMTLPCMYDQCKHMLYVSSELHRLQVSYEEYLCMKTLLLLSSVPKDGLKSQELFDEIRMTYIKELGKA
IVKREGNSSQNWQRFYQLTKLLDSMHEVVENLLNYCFQTFLDKTMSIEFPEMLAEIITNQIPKYSNGNIK
KLLFHQK
```

```
# sp|P04150.1|GCR_HUMAN Length: 777
# sp|P04150.1|GCR_HUMAN Number of predicted TMHs:  0
# sp|P04150.1|GCR_HUMAN Exp number of AAs in TMHs: 1.55998
# sp|P04150.1|GCR_HUMAN Exp number, first 60 AAs:  0.0006
# sp|P04150.1|GCR_HUMAN Total prob of N-in:        0.00014
sp|P04150.1|GCR_HUMAN   TMHMM2.0        outside      1   777
```

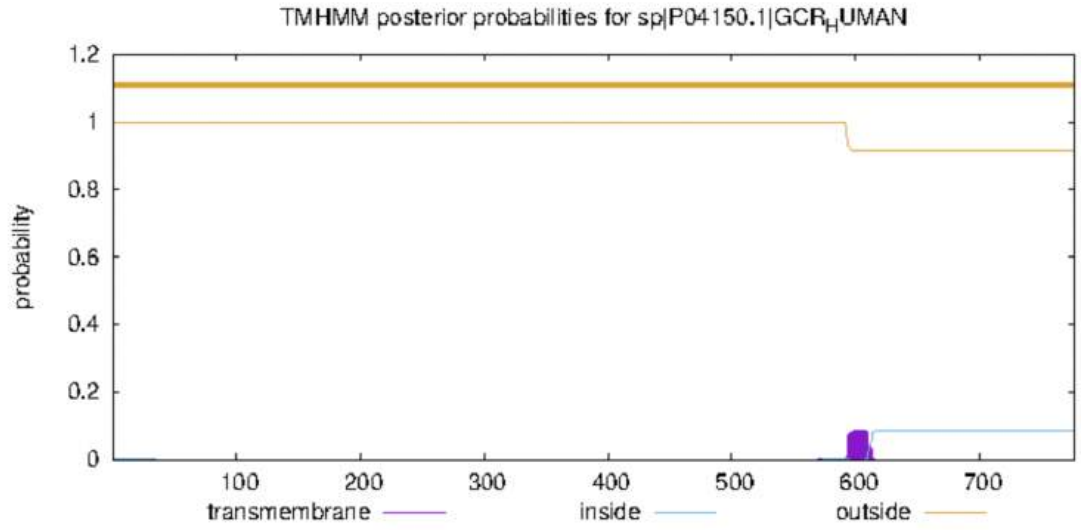


Figure 1. Transmembrane domain analysis of NR3C1. The graph shows the TMHMM prediction for the NR3C1 protein. The upper text shows the complete 777-amino-acid sequence of the NR3C1 protein retrieved from NCBI>Protein database. The x-axis represents the amino acid position, and the y-axis represents the predicted probability. The yellow line shows the probability that the protein is located outside the membrane, the blue line shows the probability of being on the other side of the membrane if there is a transpassing happening, and the purple signal shows the probability of a transmembrane region. No strong purple peak is present, and TMHMM predicted zero transmembrane helices. This indicates that NR3C1 does not pass through the cell membrane.

3.2. Multiple protein alignment for the NR3C1 protein showed that NR3C1 is highly conserved among human, chimpanzee, pig and mouse

The NR3C1 protein sequences from human, chimpanzee, pig and mouse were compared using Clustal Omega. The program arranged the sequences so that identical or similar amino acids were placed in the same columns. The alignment showed that many parts of NR3C1 are conserved across all four species (Figure 2). Humans and chimpanzees had the most similar sequences, while pig and mouse showed more differences and gaps. The beginning of the protein sequence showed more differences between the species, while the middle and end of the sequence were more highly conserved. This is important because conserved regions are likely to have important roles in the structure or function of NR3C1 and have therefore changed very little during evolution.

```
Mouse_sp|P06537.2|GCR_MOUSE     MDSKESLAPPGRDEVPSSLLGRGRGSVMDLYKTLRGGATVKVSASSPSVAAASQADSKQQ   60
Pig_AGH25605.1                  MDPKESLTPPSREEIPSSVLGRERAHVMDFYKSLRGGTPVKVSAASPSLAAVSQPDSKQQ   60
Human_sp|P04150.1|GCR_HUMAN     MDSKESLT-PGREENPSSVLAQERGDVMDFYKTLRGGATVKVSASSPSLAVASQSDSKQR   59
Chimpanzee_XP_063667618.1       MDSKESLT-PGREENPSSVLAQERGNVMDFYKTLRGGATVKVSASSPSLAVASQSDSKQR   59
                                ** ****: *.*:* ***:*.: *. ***:**:****: *****:***:*..** ****:

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Pig_AGH25605.1                  RLAVDFPKGSGSNAQ-----------------QPDLSKAVSLSMGLYMGETETKVMGSDL  103
Human_sp|P04150.1|GCR_HUMAN     RLLVDFPKGSVSNAQ-----------------QPDLSKAVSLSMGLYMGETETKVMGNDL  102
Chimpanzee_XP_063667618.1       RLLVDFPKGSVSNAQ-----------------QPDLSKAVSLSMGLYMGETETKVMGNDL  102
                                *: :** *** ****                ************************.**

Mouse_sp|P06537.2|GCR_MOUSE     GYPQQGQLGLSSGETDFRLLEESIANLNRSTSRPENPKSSTPAAGCATPTEKEFPQTHSD  180
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Human_sp|P04150.1|GCR_HUMAN     GFPQQGQISLSSGETDLKLLEESIANLNRSTSVPENPKSSASTAVSAAPTEKEFPKTHSD  162
Chimpanzee_XP_063667618.1       GFPQQGQISLSSGETDLKLLEESIANLSRSTSVPENPKSSASTAVSAAPTEKEFPKTHSD  162
                                *:*****:.*******::*********.**** *******: :* .*:*:** **:****

Mouse_sp|P06537.2|GCR_MOUSE     PSSEQQNRKSQPGTNGGSVKLYTTDQSTFDI----LQDLEFSAGSPGKETNESPWRSDLL  236
Pig_AGH25605.1                  GAPEQPNVKGQTGTNGGNVKLFTTDQSTFDIWRKKLQDLELPSGSPGKETSESPWSSDLL  223
Human_sp|P04150.1|GCR_HUMAN     VSSEQQHLKGQTGTNGGNVKLYTTDQSTFDI----LQDLEFSSGSPGKETNESPWRSDLL  218
Chimpanzee_XP_063667618.1       VSSEQQHLKGQTGTNGGNVKLYTTDQSTFDI----LQDLEFSSGSPGKETNESPWRSDLL  218
                                 : ** : *.* *****.***:*********    *****: :*******.**** ****
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Mouse_sp|P06537.2|GCR_MOUSE      IDEN-LLSPLAGEDDPFLLEGDVNEDCKPLILPDTKPKIQDTGDTILSSPSSVALPQVKT  295
Pig_AGH25605.1                   IDENCLLSPLAGEEDPFLLEGSSTEDCKPLVLPDTKPKVKDNGELILPSPNSVPLPQVKT  283
Human_sp|P04150.1|GCR_HUMAN      IDENCLLSPLAGEDDSFLLEGNSNEDCKPLILPDTKPKIKDNGDLVLSSPSNVTLPQVKT  278
Chimpanzee_XP_063667618.1        IDENCLLSPLAGEDDSFLLEGNSNEDCKPLILPDTKPKIKDNGDLVLSSPSNVTLPQVKT  278
                                 **** ********:* *****. .******:*******::*.*: :* **..* ******

Mouse_sp|P06537.2|GCR_MOUSE      EKDDFIELCTPGVIKQEKLGPVYCQASFSGTNIIGNKMSAISVHGVSTSGGQMYHYDMNT  355
Pig_AGH25605.1                   EKEDFIELCTPGVIKQEKLGPAYCQASFSGANIIGGKMSAISVHGVSTSGGQLYHYDMNT  343
Human_sp|P04150.1|GCR_HUMAN      EKEDFIELCTPGVIKQEKLGTVYCQASFPGANIIGNKMSAISVHGVSTSGGQMYHYDMNT  338
Chimpanzee_XP_063667618.1        EKEDFIELCTPGVIKQEKLGTVYCQASFPGANIIGNKMSAISVHGVSTSGGQMYHYDMNT  338
                                 **:***************** .****** *:****.***************:*******

Mouse_sp|P06537.2|GCR_MOUSE      A-SLSQQQDQKPVFNVIPPIPVGSENWNRCQGSGEDNLTSLGAMNFAGRSVFSNGYSSPG  414
Pig_AGH25605.1                   AASLSKQQEQKPLFNVIPPIPVGSENWNRCQGSGDDNLTSLGTLNFSGRSVFSNGYSSPG  403
Human_sp|P04150.1|GCR_HUMAN      A-SLSQQQDQKPIFNVIPPIPVGSENWNRCQGSGDDNLTSLGTLNFPGRTVFSNGYSSPS  397
Chimpanzee_XP_063667618.1        A-SLSQQQDQKPIFNVIPPIPVGSENWNRCQGSGDDNLTSLGTLNFPGRTVFSNGYSSPS  397
                                 * ***:**:***:**********************:*******::** **:*********.

Mouse_sp|P06537.2|GCR_MOUSE      MRPDVSSPPSSSS-TATGPPPKLCLVCSDEASGCHYGVLTCGSCKVFFKRAVEG-QHNYL  472
Pig_AGH25605.1                   MRPDVSSPPSSSS-AATGPPPKLCLVCSDEASGCHYGVLTCGSCKVFFKRAVEGRQHNYL  462
Human_sp|P04150.1|GCR_HUMAN      MRPDVSSPPSSSSTATTGPPPKLCLVCSDEASGCHYGVLTCGSCKVFFKRAVEG-QHNYL  456
Chimpanzee_XP_063667618.1        MRPDVSSPPSSSSTATTGPPPKLCLVCSDEASGCHYGVLTCGSCKVFFKRAVEG-QHNYL  456
                                 ************ ::************************************ *****

Mouse_sp|P06537.2|GCR_MOUSE      CAGRNDCIIDKIRRKNCPACRYRKCLQAGMNLEARKTKKKIKGIQQATAGVSQDTSEN-A  531
Pig_AGH25605.1                   CAGRNDCIIDKIRRKNCPACRYRKCLQAGMNLEARKTKKKIKGIQQATTGVSQETSENSA  522
Human_sp|P04150.1|GCR_HUMAN      CAGRNDCIIDKIRRKNCPACRYRKCLQAGMNLEARKTKKKIKGIQQATTGVSQETSENPG  516
Chimpanzee_XP_063667618.1        CAGRNDCIIDKIRRKNCPACRYRKCLQAGMNLEARKTKKKIKGIQQATTGVSQETSENPA  516
                                 ************************************************:****:**** .

Mouse_sp|P06537.2|GCR_MOUSE      NKTIVPAALPQLTPTLVSLLEVIEPEVLYAGYDSSVPDSAWRIMTTLNMLGGRQVIAAVK  591
Pig_AGH25605.1                   NKTIVPATLPQLTPTLVSLLEVIEPEVLYAGYDSSIPDSTWRIMTALNMLGGRQVIAAVK  582
Human_sp|P04150.1|GCR_HUMAN      NKTIVPATLPQLTPTLVSLLEVIEPEVLYAGYDSSVPDSTWRIMTTLNMLGGRQVIAAVK  576
Chimpanzee_XP_063667618.1        NKTIVPATLPQLTPTLVSLLEVIEPEVLYAGYDSSVPDSTWRIMTTLNMLGGRQVIAAVK  576
                                 *******:*****************************:***:*****:***************

Mouse_sp|P06537.2|GCR_MOUSE      WAKAIPGFRNLHLDDQMTLLQYSWMFLMAFALGWRSYRQASGNLLCFAPDLIINEQRMTL  651
Pig_AGH25605.1                   WAKAIPGFRNLHLDDQMTLLQYSWMFLMAFALGWRSYRQSSASLLCFAPDLVINEQRMAL  642
Human_sp|P04150.1|GCR_HUMAN      WAKAIPGFRNLHLDDQMTLLQYSWMFLMAFALGWRSYRQSSANLLCFAPDLIINEQRMTL  636
Chimpanzee_XP_063667618.1        WAKAIPGFRNLHLDDQMTLLQYSWMFLMAFALGWRSYRQSSANLLCFAPDLIINEQRMTL  636
                                 ************************************:*..********:******:*

Mouse_sp|P06537.2|GCR_MOUSE      PCMYDQCKHMLFISTELQRLQVSYEEYLCMKTLLLLSSVPKEGLKSQELFDEIRMTYIKE  711
Pig_AGH25605.1                   PCMYDQCRHMLYVSSELQRLQVSYEEYLCMKTLLLLSSVPKDGLKSQELFDEIRMTYIKE  702
Human_sp|P04150.1|GCR_HUMAN      PCMYDQCKHMLYVSSELHRLQVSYEEYLCMKTLLLLSSVPKDGLKSQELFDEIRMTYIKE  696
Chimpanzee_XP_063667618.1        PCMYDQCKHMLYVSSELHRLQVSYEEYLCMKTLLLLSSVPKDGLKSQELFDEIRMTYIKE  696
                                 *******:***::*:**:**********************:******************

Mouse_sp|P06537.2|GCR_MOUSE      LGKAIVKREGNSSQNWQRFYQLTKLLDSMHDVVENLLSYCFQTFLDKSMSIEFPEMLAEI  771
Pig_AGH25605.1                   LGKAIVKREGNSSQNWQRFYQLTKLLDSMHDVVENLLNYCFQTFLDKNMSIEFPEMLAEI  762
Human_sp|P04150.1|GCR_HUMAN      LGKAIVKREGNSSQNWQRFYQLTKLLDSMHEVVENLLNYCFQTFLDKTMSIEFPEMLAEI  756
Chimpanzee_XP_063667618.1        LGKAIVKREGNSSQNWQRFYQLTKLLDSMHEVVENLLNYCFQTFLDKTMSIEFPEMLAEI  756
                                 ******************************:******.*********.************

Mouse_sp|P06537.2|GCR_MOUSE      ITNQIPKYSNGNIKKLLFHQK  792
Pig_AGH25605.1                   ITNQLPKYSSGNIKKLLFHQK  783
Human_sp|P04150.1|GCR_HUMAN      ITNQIPKYSNGNIKKLLFHQK  777
Chimpanzee_XP_063667618.1        ITNQIPKYSNGNIKKLLFHQK  777
                                 ****:****.***********
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Figure 2. Multiple protein alignment for NR3C1. The figure shows the aligned NR3C1 amino acid sequences from human, chimpanzee, pig and mouse. Each row represents the protein sequence from one species, and the numbers on the right show the amino acid position. The amino acids are displayed in different colours according to their chemical properties. Red indicates positively charged amino acids, magenta indicates negatively charged amino acids, blue indicates hydrophobic amino acids and green indicates polar amino acids. An asterisk (*) indicates that the same amino acid is present in all four sequences. A colon (:) indicates amino acids with strongly similar properties, while a full stop (.) indicates weaker similarity. Dashes represent gaps added by Clustal Omega to produce the best alignment.

3.3. STRING Protein database generated a predicted protein-protein interaction map for NR3C1

The NR3C1 protein was analysed using the STRING Protein database to identify proteins that are known or predicted to interact with it. In the network, each circle represents a protein, and the lines between the circles represent functional associations supported by different types of evidence. Several colours can appear in one connection between two protein nodes because more than one type of evidence may support the same association. NR3C1 has several predicted interactors (Figure 3): HSP90AA1, HSP90AB1, HSPA4, FKBP4, FKBP5, STIP1, PTGES3, NCOA1, NCOA2 and NCOR1. Their roles are mainly related to receptor folding, stability and gene regulation. HSP90AA1 and HSP90AB1 act as molecular chaperones that support the maturation, structural maintenance and regulation of target proteins. FKBP5 is a co-chaperone involved in unliganded steroid receptor complexes and helps keep these complexes in the cytoplasm. FKBP4 is also a co-chaperone and may help transport steroid receptor complexes between the cytoplasm and nucleus. NCOA1 and NCOA2 are nuclear receptor coactivators that stimulate hormone-dependent transcription, while NCOR1 acts as a corepressor that reduces transcription. PTGES3 functions as a prostaglandin synthase and molecular chaperone and can promote the disassembly of receptor-mediated transcription complexes. STIP1 connects HSP70-family chaperones with HSP90, and HSPA4 is a member of the heat-shock protein 70 family.

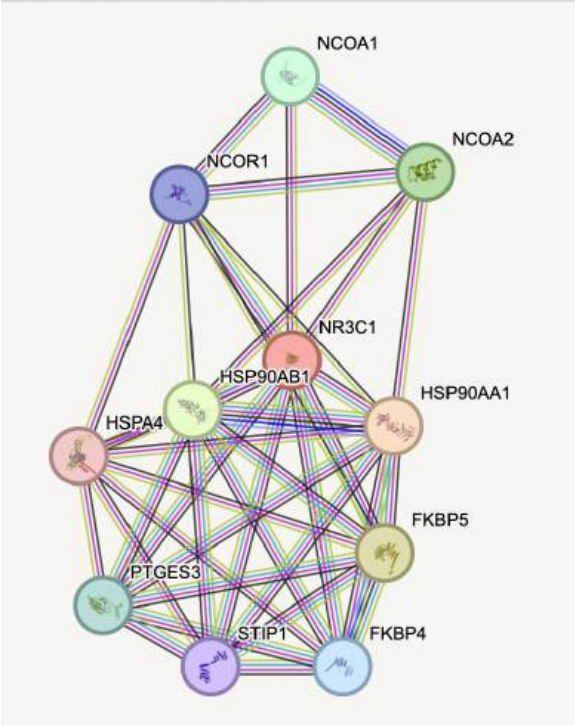


Figure 3. Protein-protein interaction map of NR3C1. The figure shows the NR3C1 interaction network generated using the STRING database. Each node represents a protein, while the lines between nodes represent known or predicted associations, and the image inside each node shows its known or predicted three-dimensional structure. The different line colours show the type of evidence supporting each association. Pink lines represent experimentally determined interactions, making them especially important because they are supported by laboratory experiments. Light blue lines represent information from curated databases, green lines represent gene neighbourhood, red lines represent gene fusion, dark blue lines represent gene co-occurrence, light green lines represent text-mining evidence, black lines represent co-expression, and purple lines represent protein homology. Several proteins are connected to NR3C1, including NCOA1, NCOA2, NCOR1, HSP90AA1, HSP90AB1, HSPA4, FKBP4, FKBP5, STIP1 and PTGES3. Multiple coloured lines between two proteins indicate that the association is supported by more than one type of evidence.

3.4 CRISPR/Cas9 gene editing design for NR3C1 with three guideRNAs

A theoretical CRISPR/Cas9 gene-editing design was created for a selected region of the NR3C1 DNA sequence. The sequence was examined to identify potential guide RNA target sites located next to protospacer-adjacent motif (PAM) sequences, which are required for Cas9 recognition and targeting. The PAM sequence must contain two guanines next to each other, and the nucleotide before them can be any of the four DNA bases. Three possible target sites were identified and labelled guide RNA 1, guide RNA 2 and guide RNA 3 (Figure 4). Each guide RNA was positioned next to its corresponding PAM sequence, indicating a possible location at which the CRISPR/Cas9 system could be directed to NR3C1. In addition to the presence of a

suitable PAM, guide RNA selection should consider how closely the guide matches the intended NR3C1 sequence. The presence of similar sequences elsewhere in the genome could lead to off-target editing. Therefore guideRNAs were chosen based on off-target scores in the CRISPR Finder database. The scores aimed were 0=1, 1=0, 2=0 and for 3 and 4 as low as possible. The three guide RNA use aimed to increase the possibility of deletion mutation to reach a knock out version of the gene but not fully removed. The design provides several possible target sites for experimental modification of NR3C1 and establishes a framework for investigating how specific genetic changes may affect glucocorticoid-receptor function and hormonal regulation.

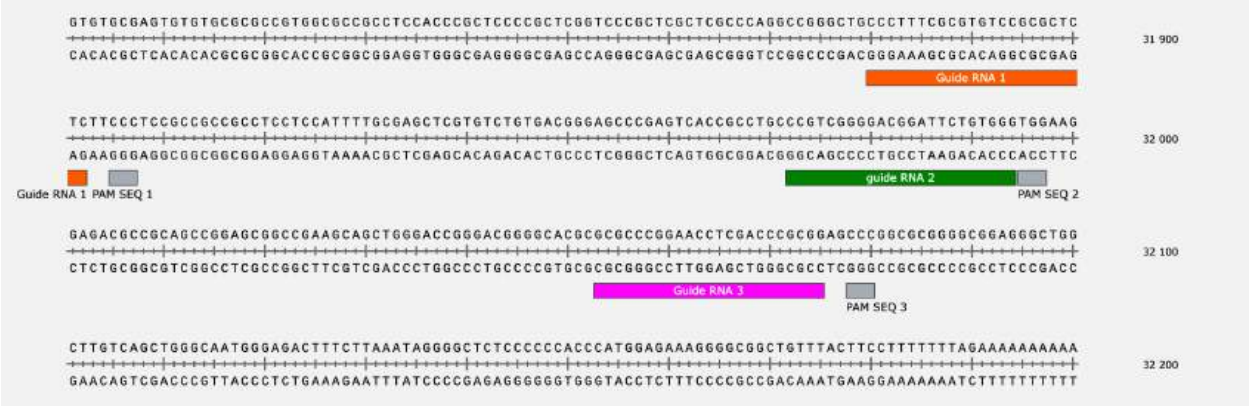


Figure 4. CRISPR/Cas9 gene editing strategy for NR3C1. The figure shows a selected region of the NR3C1 DNA sequence with three potential CRISPR/Cas9 target sites. Guide RNA 1 is displayed in orange, guide RNA 2 in green and guide RNA 3 in pink. The smaller labelled boxes indicate the PAM sequence associated with each guide RNA. The nucleotide numbers on the right show the approximate positions of the displayed sequence. The design identifies possible sites for directing Cas9 to NR3C1 but has not been experimentally validated.

4. Discussion

The findings suggest that NR3C1 acts as a central link between cortisol signalling, regulation of gene expression and the biological effects of stress. The protein properties analyses support this role from several directions. NR3C1 is an intracellular receptor rather than a membrane protein, its sequence contains strongly conserved regions, and it is connected to proteins involved in receptor stability, transport and transcriptional control. Together, this indicates that the glucocorticoid response depends on a preserved receptor structure and on a group of supporting proteins that allow the receptor to function correctly.

Epigenetic changes are another way the body can control NR3C1 and the stress response. Stress does not need to alter the NR3C1 protein sequence to affect its function. Instead, changes in methylation near NR3C1 may influence how much glucocorticoid receptor is produced. Because this receptor participates in feedback regulation of the HPA axis, altered expression may change how the body controls cortisol after stress. The findings on prenatal stress, maternal

depression and early-life adversity therefore suggest a pathway in which life experiences influence epigenetic regulation, which may then affect hormonal stress responses.

The overall interpretation is that NR3C1 is regulated at several connected levels. Its conserved sequence supports its basic function, its interacting proteins help control receptor activity and epigenetic modifications may alter its expression. Disruption at any of these levels could weaken or change glucocorticoid signalling. This helps explain why both genetic mutations and environmentally associated methylation changes have been linked to altered cortisol regulation, psychiatric conditions and impaired hormonal feedback.

NR3C1 shows why stress-related conditions cannot be explained by genetics alone. The same DNA sequence can be regulated differently depending on environmental exposure, developmental stage, and epigenetic changes. This may help explain why people exposed to similar stressful experiences do not always develop the same hormonal or psychological outcomes. It also highlights the importance of studying stress across several biological levels at the same time. The nervous system detects and responds to stress, the endocrine system controls cortisol release through the HPA axis and epigenetic mechanisms can influence how strongly genes such as NR3C1 are expressed. These systems are closely connected rather than acting separately, so changes in one part of the pathway may affect the others and alter the overall stress response.

A major limitation of this study is that it was conducted entirely through online databases and computational tools. No laboratory experiments were performed to confirm the results. Therefore, the findings are theoretical and should be interpreted as predictions rather than direct experimental evidence. For example, the proposed CRISPR/Cas9 gene-editing design was not carried out in cells or an experimental organism. Its practical efficiency, accuracy and effects on NR3C1 function could therefore not be evaluated.

The protein analyses were also limited by the information available in the selected databases and bioinformatics tools. Predictions concerning transmembrane regions, sequence conservation and protein interactions depend on the data and algorithms used by these resources. Some biological and clinical data may not be publicly available or may not yet have been added to the databases. Consequently, the analyses may not represent every known NR3C1 variant, interaction or functional effect.

For future studies to follow up this research, it would be useful to compare different types of stress and the stages of life in which they occur, since prenatal stress, early-life adversity, and later trauma may influence NR3C1 in different ways. Prenatal stress and early-life adversity were often associated with increased NR3C1 methylation, which may reduce glucocorticoid-receptor expression and contribute to altered cortisol regulation. In contrast, later traumatic experiences and post-traumatic stress disorder were associated with reduced NR3C1 methylation, which may increase receptor expression and sensitivity of the HPA axis.

The field should investigate how epigenetic mechanisms, including DNA methylation and histone modifications, affect genes involved in hormone production, hormone receptors and nervous-system function. Since the nervous and endocrine systems are closely connected, future

research should examine several biological levels at the same time rather than studying each system separately. Another important focus should be the long-term effects of epigenetic changes. Studies should examine whether environmentally associated modifications remain stable, change over time or can be reversed. Researchers should also combine measurements of epigenetic regulation, hormone levels, receptor expression and nervous-system responses to better understand how daily life and stressful experiences influence hormonal regulation.

5. Conclusion

This study examined how daily life and stressful experiences may influence nervous-system-mediated hormonal regulation through epigenetic mechanisms, using NR3C1 as a central candidate. NR3C1 encodes the glucocorticoid receptor, which contributes to regulation of the HPA axis and the body's cortisol response. The main conclusion is that daily experiences, especially stress, may influence hormonal regulation through epigenetic changes without altering the underlying DNA sequence. Instead, stress may be associated with changes in DNA methylation that influence glucocorticoid-receptor expression and hormonal feedback. The protein analyses further supported the role of NR3C1 as an intracellular receptor involved in gene regulation. NR3C1 had no predicted transmembrane region, contained conserved regions across several species and was connected to proteins involved in receptor folding, stability, transport and transcriptional control. Together, these findings show that hormonal stress regulation depends on interactions between environmental exposure, epigenetic regulation, protein function, glucocorticoid signalling and the HPA axis.

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Autism Spectrum Disorder and Oral Health: A Multidimensional Review of Contributing Factors By Joonsuh Park[1]

Abstract

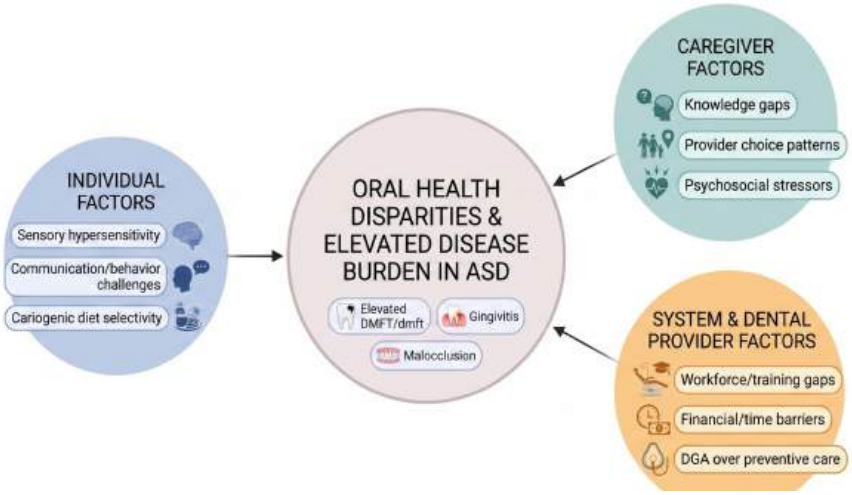
Autism spectrum disorder (ASD) is a neurobehavioral condition characterized by difficulties in social interactions, with restricted, repetitive patterns of behavior, interests, or activities. Moreover, ASD has a significant impact on oral health. The purpose of this comprehensive literature review is to examine how factors of individuals with ASD, their caregivers, and oral health providers influence the overall oral health of patients with ASD, and how these factors influence differences in dental treatment and outcomes between ASD children and typically developing peers. Sensitivity to auditory, gustatory, and visual stimuli are increased in ASD and these factors influence oral hygiene practices. ASD children exhibit difficulty in expressing issues and pain, making communication and behavioral factors a key factor in their care. Additionally, diet and different pharmacologic interactions from medications affect their oral health. ASD children have a significantly higher consumption of high sugar products, leading to a higher number of DMFT. Also, the medicines that patients with ASD consume affect oral health. Other factors include caregiver's knowledge, and systemic barriers in dental offices, possibly due to increased emotional sensitivity and a lack of combined effort between parents and caregivers to work together to create a plan for care in ASD children. This article comprehends the challenges presented by ASD children emphasizing on broader systemic changes to improve the care of ASD children.

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by persistent challenges in social communication and interaction, alongside restricted, repetitive patterns of behavior, interests, or activities (1). Symptoms typically emerge in early childhood, often within the first three years of life, with some indicators observable as early as 18 months of age (2). Early diagnosis is essential because timely interventions could improve long-term developmental, behavioral, and functional outcomes (3). Clinically, ASD represents a heterogeneous spectrum of symptom severity, adaptive functioning, and support needs. Individuals with ASD are reported to experience challenges with reciprocal social interaction, including difficulty initiating or continuing conversation, reduced sharing of interests or emotions, and impaired use of interpretation of nonverbal communicative behaviors, such as eye contact, facial expression, and body language. In addition, some behaviors are manifested as stereotyped motor movements, inflexible adherence to routines, or highly circumscribed interests of unusual intensity or focus.

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Figure 1: Tripartite Model of Contributing Factors to Oral Health Disparities in ASD



Historically, autism was classified into distinct diagnostic subtypes, including "autistic disorder," "Asperger disorder", and "pervasive developmental disorder". However, *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5), defined these classifications into a single diagnosis of "Autism Spectrum Disorder" (1). DSM-5 helps to identify ASD at an earlier age. The DSM-5 framework emphasizes that ASD is defined along a spectrum reflecting varying levels of functional support needs, rather than being categorized into rigid subtypes. The three major domains for diagnostic criteria in the DSM-V include (a) social-emotional reciprocity, (b) deficits in nonverbal communication including eye-contact, unusual interest in sensory aspects of the environment, and repetitive behavior, and (c) difficulties in developing, maintaining, or understanding social relationships. Repetitive behavior has been characterised as stereotyped or repetitive motor movements, inflexible adherence to routines, and highly restricted, fixated interests (1). Contemporary models increasingly reject simplistic distinctions such as "high-functioning" or "low-functioning," which inadequately capture the complexity of ASD (4). Instead, multiple studies proposed a multidimensional framework, often illustrated through a color wheel, where different traits such as communication, sensory processing, cognitive flexibility, and emotional regulation, vary in intensity across individuals (5). This framework highlights that individuals with the same diagnosis may present with markedly different strengths, challenges, and clinical priorities, underscoring the importance of individualized assessment and intervention.

Globally, ASD is increasingly recognized as a significant public health concern. The prevalence of ASD has steadily increased over recent decades, largely due to improved awareness, refined diagnostic tools, and better reporting systems (6). According to the 2010 Global Burden of Disease study, approximately 52 million people were living with autism worldwide (7). A systematic review by Elsabbagh et al; estimated the global median prevalence at 62 per 10,000 children, while an updated meta-analysis by Zeidan et al; reported a higher prevalence of 119.25 per 10,000 individuals apart from regional variation. Prevalence has been

estimated at 86.5 per 10,000 in the Eastern Mediterranean and 203.1 per 10,000 in the Western Pacific (8,9). These differences reflect true epidemiologic variation in research methodology, diagnostic capacity, and healthcare infrastructure. In the United States, the Centers for Disease Control and Prevention (CDC) report that approximately 1 in 59 children (1.68%) aged 8 years are identified with ASD, with parent-reported surveys suggesting even higher prevalence around 2.5% (10,11). Males are constantly diagnosed more frequently than females, with ratios ranging from 2:1 to 5:1, although emerging research suggests that autism in females may be underdiagnosed due to different behavioral phenotypes and camouflaging strategies (12).

Beyond behavioral and developmental aspects, ASD has significant implications for oral health. Individuals with autism consistently demonstrate compromised oral health outcomes compared to neurotypical peers (13). Barriers to optimal health are multifactorial and include sensory sensitivities, behavioral challenges, communication limitations, and systemic barriers to care. Sensory aversions to overbearing stimuli make routine dental visits distressing, while difficulties with cooperation and sustained attention complicate clinical management. Complications such as limited verbal expression, delays recognition of dental pain. Studies have reported increased rates of dental caries, gingivitis, and periodontal disease in both children and adults with autism (14). A recent meta analysis demonstrated that individuals with ASD are at significantly higher risk for gingivitis, bruxism, and several malocclusions including overjet, overbite, cross bite, and open bite (13). Caries prevalence is particularly elevated in primary dentition, often linked to selective eating behaviors and dietary preferences characterized by frequent consumption of fermentable carbohydrates, sugary snacks, and acidic beverages. Also, the consequences of poor oral health lead to issues with speech development, sleep quality, and social interactions, thereby compounding the daily challenges already faced by individuals with autism and caregivers. Furthermore, poor oral health has been linked to systemic inflammation, underscoring the importance of addressing oral health disparities in ASD population (15).

Given these disparities, there is a critical need for research that examines oral health outcomes, contributory factors, and barriers to dental care among individuals with ASD across diverse populations. Improved understanding of these factors may lead to the development of targeted strategies to reduce inequities, enhance care, and promote overall well-being. The purpose of this article is to examine key individual, caregiver, and provider level factors influencing oral health status among individuals with ASD to discuss how these factors contribute to disparities in dental treatment and outcomes.

Materials and Methods

Study Design and Research Question

This study is a literature review designed to examine factors that influence the oral health of individuals with ASD. The review was guided by a structured research question and PICO framework to ensure proper analysis. The guiding research question was: In patients with ASD, what factors affect oral health of patients?

PICO Framework:
P- Patients with ASD
I- Factors affecting oral health of patients
C- None
O- Oral Health outcomes

Data Sources and Search Strategy

A comprehensive search was conducted using PubMed and Google Scholar. Peer-reviewed journal articles and empirical studies were considered. Search terms included combinations of "ASD," "Autism Spectrum Disorder," "Oral Health," “children,” “communication,” “sensory processing,” “medication,” “behavior,” and “factors”. The review focuses on sources published between 2008 and 2025, with particular emphasis on studies examining contributors to oral health challenges among individuals with ASD.

Inclusion criteria: “Autism Spectrum Disorder,” “Oral Health,” “Children”
Exclusion criteria: “Abstract” “Before 2000” “Books”

Discussion

ASD Severity

Historically, autism severity has been informally conceptualized on the basis of IQ, level of language acquisition/functioning, and severity of behavioral problems. These indicators have been used to stratify individuals for research purposes and to provide prognostic information to families and educators. Currently, in children with ASD, severity is generally conceptualized in terms of language deficits, cognitive impairment, adaptive behavior impairment, and the presence of challenging behavior, such as aggression. Subsequently, ASD has been classified based on the level of intellectual functioning as a proxy for severity. However, universally accepted methods are currently unavailable for scoring the relative contribution of these variables when determining overall severity for clinical or research purposes. The DSM-5 offers the opportunity to distinguish between various subsets of children with ASD by assigning ‘‘severity ratings’’ based on the ‘‘severity of support needs’’ separately for both social communication and restricted/repetitive behavior symptom categories. The general contributing factors and impact of ASD in oral health care are given in **Table 1**.

Table 1: Contributing factors and impact of ASD in oral health care.

Dimension	Specific Contributing Factors	Direct Impact on Oral Health & Care	Citation

Individual Level	• **Sensory Processing Differences:** Tactile/auditory hypersensitivity (e.g., aversion to toothbrush vibrations, light touch, clinic noises). • **Communication Barriers:** Difficulty expressing dental discomfort or pain verbally. • **Behavioral Challenges:** Tantrums, aggression, or self-injurious actions during routines. • **Dietary Preferences:** High selectivity favoring soft, sweet, or fermentable carbohydrates. • **Medical Comorbidities:** High rates of co-occurring ADHD or epilepsy/seizures.	• Resistance to daily brushing/flossing. • Delayed detection of dental decay/pathology. • Significantly increased risk for caries and gingivitis. • Uncooperative behavior during clinical evaluations, leading to reliance on sedation.	AlHumaid et al., 2020; Cermak et al., 2015; Alghafis et al., 2023
Caregiver Level	• **Practical Knowledge Gaps:** Misunderstanding correct brushing durations (2 mins) and frequencies. • **Provider Misalignment:** Relying heavily on general pediatricians or general dentists over special-needs specialists. • **Psychosocial Stressors:** Chronic emotional toll, including higher vulnerability to divorce in late childhood.	• Suboptimal home hygiene execution. • Lack of early preventive dental tracking. • Fragmented daily routines and reduced capacity to manage intensive behavior modification for oral care.	AlHumaid et al., 2020; Teste et al., 2026
System & Provider Level	• **Workforce Deficiencies:** Lack of clinical training, confidence, and preparedness among dentists regarding special needs populations. • **Financial Barriers:** Insufficient reimbursement/compensation for the extended appointment times required. • **Implicit Biases:** Micro-aggressive attitudes, clinical preconceptions, and rigid clinical frameworks.	• Reluctance or outright refusal by general practitioners to treat individuals with ASD. • Over-reliance on invasive Dental General Anesthesia (DGA) rather than desensitization. • Heightened feelings of stigma reported by families.	Como et al., 2021; Al Ghafis et al., 2023

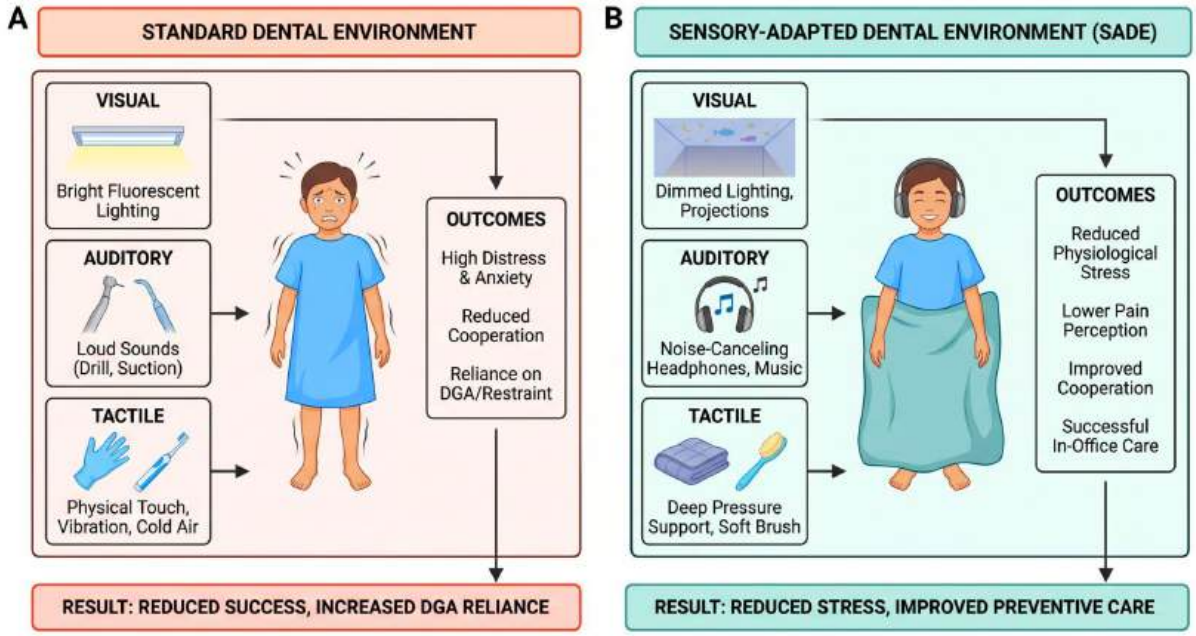
Sensory Processing Differences and Oral Health

Sensory processing differences are one of the important factors that influence the oral health of patients with ASD accounting up to 95% (16). Increased sensitivity to tactile, auditory, gustatory, and visual stimuli adversely influence oral hygiene practices and dental care

utilization. Aversion to toothbrushing and selective dietary preferences favoring foods high in sugar, fat, or salt are common challenges among children with ASD resulting in poor oral hygiene behaviors. Approximately 22.7% of such ASD subjects require assistance with toothbrushing or failing to brush (17).

According to the behavioral and sensory assessments, evidence indicates that typically developing (TD) peers have lowered sensory sensitivity compared to children with ASD. When behavioral ratings were separated into Relaxed/Cooperative (0-2) vs. Stressed (3-5), TD children showed a higher rate of 91% for relaxed and 96% for cooperative. Notably the behavioral ratings were between 0-2 for both routine dental environments and sensory-adapted dental environments. In the responses among children with ASD, there were larger gaps in behavior responses (16). For ASD children, 46% were rated as relaxed and cooperative in a regular dental environment. Notably, implementation of a sensory adapted dental environment to reduce distress, discomfort, and perception of pain during oral prophylaxis, 59% of ASD children rated as relaxed and cooperative, showing a measurable increase. This suggests that environmental sensory modifications reduce the stress level in this group. These findings underscore the heightened sensory reactivity characteristic of ASD and highlight the potential benefit of structured sensory accommodations in clinical settings (16).

Figure 2: Standard Dental Clinic vs Sensory-Adapted Dental Environment (SADE)



Alterations in oral health indices demonstrate disparities between children with ASD and neurotypical controls. Higher values for DMFT index (permanent dentition) and the dmft index (primary dentition) are shown in boys with ASD compared to the control group. DMFT is a scoring index that counts the number of teeth that are decayed, missing, and filled. In the study by Prynda et al, 32.76% in the group of ASD had DMFT/dmft values above 5 (23) whereas

9.43% of boys in the control group exhibited such DMFT/dmft values (23). While 10.34% of the group demonstrated a DMFT value higher than 5; however, no boy in the control group exhibited DMFT values above 5(23). These findings suggest a disproportionate burden of untreated or previously treated dental disease among children with ASD. Inequalities in access and preventive oral health are shown in patterns of dental service utilization. Compared to their neurotypical counterparts, the frequency of children with ASD attending dental appointments is lower. In contrast, 12.16% ASD children of the group failed in dental appointments at least once a year; while, all children in the control group attended a dental appointment (23). A combination of sensory aversions, behavioral challenges, caregiver burden, and limited availability of autism-competent dental services reflect reduced attendance.

Sensory processing differences, especially tactile and auditory hypersensitivity, plays an important role in shaping oral health behaviors and dental care experiences in ASD. Even though tactile sensitivity is one of the important clinical observations despite limited attention from researchers. Clinically, ASD patients show a dislike of light touch to the head and body. Vibrotactile stimuli are used by psychophysical tactile studies to measure the thresholds and sensitivity. Adults with ASD showed lower tactile perceptual thresholds for 200Hz but not 30Hz vibrotactile stimuli, implying a specific hypersensitivity in the Pacinian corpuscles receptor pathway (19). Tactile hypersensitivity was again shown to vibrotactile stimuli as well as thermal stimuli, but not to light touch in adults with autism. However, no tactile perceptual threshold differences for vibrotactile (40 Hz, 250 Hz) are shown among a small cohort of children with ASD (19). Daily oral health practices are affected by these sensory differences. From the date, more than half of the participants with ASD (54.6%) required active assistance with tooth brushing with only 13.8% brushing their teeth independently (20). The comparison of behavioral and clinical data metrics between individuals with ASD and neurotypical controls is summarized in Table 2.

Table 2: Behavioral and clinical data metrics comparing ASD and neurotypical/control populations.

Assessment Category	**Metric / Variable**	**ASD Population Data**	**Neurotypical (TD) / Control Data**
Clinical Oral Disease Burden	**DMFT/dmft Score > 5**	• **32.76%** (Primary) • **10.34%** (Permanent)	• **9.43%** (Primary) • **0.00%** (Permanent)
Dental Attendance	**Missed Appointments**	**12.16%** fail to attend annually	**0.00%** failed to attend
Home Hygiene Autonomy	**Toothbrushing Dependence**	• **13.8%** independent • **54.6%–70.7%** require active assistance	*High developmental independence*

Clinical Behavior Ratings	**Relaxed & Cooperative (0–2)**	• **46%** in standard clinic • **59%** in sensory-adapted clinic	**91%** (Relaxed) / **96%** (Cooperative)
Home Hygiene Adherence	**Hygiene & Dietary Flaws**	• **22.7%** do not brush • **61.3%** do not floss • **24.0%** high-sugar diet	*Standard baseline*
Clinical Management	**Treatment Pathway**	**67.1%** past visits & **78%** current hospital visits require **General Anesthesia (DGA)**	*Standard behavior management*

Auditory processing differences further compound challenges in dental environments. Low-level auditory stimuli (e.g., pitch discrimination) deficits vary widely within ASD, with some interesting differences when low- and high-functioning children (based on IQ assessment) are compared. For instance, people with ASD have problems in attention and sensory processing, and some high-functioning ASD individuals have enhanced perceptual abilities (21). ASD individuals seem better at detecting additional unexpected and expected sounds at several acoustic parameters, suggesting increased auditory perceptual capacity. A possible reason for this finding is because of the relationship with C-fiber and neural mechanism involvement. However, this heightened sensitivity often coexists with impairments in sound discrimination, particularly in noisy environments (21). Difficulties in the processing of sensory stimuli have been confirmed by electrophysiology and brain imaging data at early stages of perceptual processing (21). This supports the hypothesis that atypical auditory and language processing trajectories may exert cascading effects on core features of ASD (21).

Collectively, these findings illustrate how sensory hypersensitivity, impaired communication, and behavioral challenges intersect to negatively influence oral hygiene behaviors, dental attendance, and overall oral health outcomes among children with ASD. They further support the need for sensory-informed, individualized approaches to both preventive oral health practices and clinical dental care.

Communication and Behavioral Factors

Communication differences significantly influence oral health outcomes among individuals with ASD. Individuals with ASD often do not respond to conventional behavior modification strategies, such as rewards for positive behavior or negative consequences for undesirable behavior, in the same manner as typically developing peers (22). Furthermore, food selectivity is a common behavioural challenge in children with developmental disabilities, resulting in a predominantly cariogenic diet and thus, increased caries risk when coupled with

other risk factors such as inadequate biofilm control (22). Individuals with ASD are often unable to communicate their oral health needs to their caregivers or their oral care providers (22). Guardians of children with ASD reported difficulty in recognizing dental pain in their children due to inhibited verbal communication skills (22). This limitation emphasizes the importance of non-verbal communication methods for both caregivers and oral health professionals when working with this population (22).

Oral Disease Burden

Common assumptions regarding heightened dental anxiety demonstrate that relatively low levels of observable anxiety during dental visits of children with ASD. To cite, only 11% of the children in a seminal study were described as anxious or shaking during dental appointments, and only four children required sedation to receive dental care (24). Although these findings were derived from observational studies rather than clinical trials, the findings suggest associations between ASD characteristics and increased susceptibility to oral health problems (25). Several contributing factors explain these associations, including atypical oral motor development, altered salivary composition, and heightened sensory defensiveness (25). These characteristics complicate routine oral hygiene practices and pose challenges during dental treatment, potentially contributing to poorer oral health outcomes over time.

Al-Humaid et al reported that among the participants, 17 individuals (22.7%) reported complete lack of brushing their teeth l, 46 (61.3%) lack floss, and 18 (24%) reported sugar intake (17). The prevalence of dental caries was high, affecting 76% of primary teeth and 68% of permanent teeth, while gingival problems were observed in 31% of participants (17). Overall, oral health status and oral health practices among the study population were inconsistent. Although the prevalence of dental caries among individuals with ASD remains a topic of debate, the findings of the present study indicate a substantial burden of disease in both primary and permanent dentitions. This high prevalence may be largely attributed to inadequate oral hygiene behaviors, as evidenced by the high proportions of participants. However, statistical analysis revealed no significant association between dental caries and reported sugar consumption or oral hygiene practices. Additionally, only 29.3% of participants were able to brush their teeth independently, underscoring the reliance on caregiver support for daily oral hygiene (17).
The challenges included the presence of tantrums, aggression, and self-injurious behaviors used to avoid toothbrushing, as well as difficulties with oral–motor skills such as spitting and maintaining appropriate posture (26). Additionally, Teste et al reported that some parents were required to physically restrain their children in order to complete toothbrushing. Other reported difficulties included heightened anxiety related to the candidate's fear of the task, challenges in engagement and attention, limited comprehension of toothbrushing procedures, and the presence of underlying medical conditions that further complicated oral hygiene routines (26).

Comorbidities and Pharmacologic Interactions

Children with comorbid conditions accounted for 41.5% of 146 children, with the most common comorbidities being attention deficit hyperactivity disorder (ADHD) and epilepsy/seizure disorders, with the rare occurrence of 25.6% (34). Hypothyroidism, asthma, Down syndrome, cleft lip, and cleft lip and palate are the less frequent conditions with the occurrence of 7.3% (34). Even though most of the children with ASD(92.7%) visited a dental clinic, the majority of them exhibited negative behavior (74.4%), and most of them exhibited negative behavior during time of presentation in the hospital (75.6%) (34).

Caregiver Oral Health Knowledge

More than 86% caregivers correctly identified the association between sugar consumption and dental caries. They recognized 'gum bleeding' as a sign of gingival inflammation, and it often results from infrequent toothbrushing (28). About 70% of caregivers agree that to prevent gingivitis, regular brushing and flossing is important. Also, the concept of dental biofilm or plaque is accurately addressed by 34.7% of caregivers (28). However, there were huge gaps in practical knowledge. The percentage of caregivers who correctly reported the frequency of toothbrushing (three times per day) and recommended brushing duration of approximately two minutes is only 8.3% and 23.6%, respectively (28). Furthermore, nearly half correctly identified both the appropriate amount of toothpaste and the recommended interval for toothbrush replacement, while 56.9% indicated that routine dental check-ups should occur every six months (28).

Compared to many parents who believed that visiting the dentist every six months is important (38.3%), only 12.6% believed that children should visit the dentist every three months (29). The majority of parents preferred a general dental clinic (55.8%) and a physician (77.2%) when children experienced dental pain. This indicates that most parents rely on medical rather than specialized care (29).

More than knowledge-based limitations, caregivers' ability to support oral health has been restricted by broader psychosocial stressors. The experience of vulnerability to divorce increased when parents have children with ASD (30). Compared to the similar rate of divorce rate between the early childhood of children with ASD, the divorce rate of late childhood exhibited a similar trend among the parents of children with ASD (30). These sustained psychosocial stressors indirectly impair caregiving capacity, consistency of daily routines, and engagement with preventive health behaviors, including oral health maintenance.

System-Level Barriers and Dental Workforce Factors

System-level barriers substantially limit access to oral healthcare for individuals with ASD. In a survey of U.S. dentists, 72.3% reported that financial compensation for treating autistic patients was inadequate and did not reflect the increased costs associated with longer appointment times required for familiarization and desensitization (31). Implicit biases within dental teams emerged as a significant concern, with themes including micro-aggressive attitudes, marginalization of individuals with special needs, and clinical preconceptions. Hyper-empathy

exhibited by autistic children, combined with parents' perceived inability to advocate for their child due to the authority attributed to medical professionals, was associated with a reduced willingness among dental teams to adapt care to special needs (32). Furthermore, the study highlighted the need for greater flexibility within dental teams to ensure that parents and caregivers feel confident in the treating clinician (32). Establishing a clear treatment plan, ensuring continuity of care, and providing well-defined referral pathways to specialist dental services were emphasized as essential components of equitable and effective care (32).

The predominant care approach was dental treatment under general anesthesia (DGA), accounting for 67.1% of previous visits and increasing to 78% of treatments delivered in the hospital setting (38). Tooth extractions and other procedures were the most frequently performed interventions, reported in 86.6%, 78%, and 65.9% of cases, respectively (34). The majority of children with moderate-to-severe ASD received treatment under general anesthesia compared with those with mild conditions (34). A statistically significant association was identified between family socioeconomic status and child behavior during previous dental visits. Interestingly, negative behavior was observed in only 53.8% of children from high-income families, compared with 76.5–80% among children from low- and middle-income families (34). Further analysis revealed a significant association between the number of DGA sessions and the severity of ASD (34).

Although the literature on ASD-specific education for dental hygienists is limited, clear gaps remain in dentists' knowledge and preparedness when providing care to the patients with special needs (33). Existing studies indicate a reluctance among dentists to treat children with autism, largely due to a lack of confidence in their educational training and clinical preparation (33). Another significant barrier to oral healthcare access for individuals with ASD lies within the oral health workforce itself (33). The knowledge, attitudes, and behaviors of oral health professionals play a critical role in shaping the comfort, compliance, and overall satisfaction of individuals with ASD and their caregivers (33). Despite the growing need for inclusive dental care, gaps remain in professional education and training (33). Although the literature addressing ASD-specific education for dental hygienists is limited, substantial deficiencies have been identified in the knowledge and preparedness of dental practitioners when treating this special care population (33). Studies consistently report a reluctance among dentists to treat children with autism, largely due to a lack of confidence in their educational preparation (33). Furthermore, research indicates that dentists feel inadequately trained during their formal dental education to work with special care populations, which subsequently reduces their likelihood of providing care to individuals with developmental disabilities (33).

Families of children with autism frequently report feelings of stigma within healthcare settings due to multiple contributing factors (32). Among the 16 studies reviewed, two identified clinician bias and unfavorable attitudes toward autistic individuals as significant concerns (32). Como et al. reported that the language used by dental practitioners to describe their experiences in providing oral healthcare to autistic individuals revealed the presence of implicit bias (32). Three key themes emerged: micro-aggressive attitudes within the dental team, the

marginalization of individuals with special needs, and clinicians' preconceptions (32). Additionally, heightened emotional sensitivity in autistic children, combined with inability of parents/caregivers to advocate for their child due to the authority attributed to healthcare professionals, was associated with a reduced willingness among dental teams to adapt care to meet special needs (32).

Conclusion

There are three major factors for causing oral health problems of patients with ASD: individual factors, caregiver and family factors, and healthcare provider/dentist factors. The critical individual factors are sensory processing differences, communication differences, cognitive differences, behavior issues, and medication and other systemic conditions. A big family factor is parental knowledge. Lastly, some providers and dentists are not specialized in ASD, such as refusal to treat patients with ASD, and a higher need for sedation or general anesthesia.

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The Prognoses of Metal-Based Nanomaterials and Their Efficacy in Regenerative Medicine

By Srinidhi Drakshapelli

Abstract

Metallic nanoparticles have gained widespread prominence for their potential in the ever-changing field of regenerative medicine, a frontier in biomedical research that offers transformative strategies for restoring the structure and function of damaged tissues. The nanoscale existence of metallic and metal-oxide nanoparticles provides them with superiority over cellular functions, with individual properties owing to variations in their chemical compositions. Silver and gold nanoparticles (AgNPs and AuNPs) are known for antimicrobial properties, scaffold reinforcement, control over stem cell differentiation, and bone tissue engineering. Magnetic nanoparticles (MNPs) are, in turn, known for their control over stem cell proliferation, and the variation of iron oxide nanoparticles (IONPs) is known for cell tracking, magnet-guided engineering, and numerous other applications. The most distinguished feature of nanoparticles is their biomimetism, which allows them to appear organic and thus be excreted through the natural pathways. Nanoparticles have immense possibilities and hold promise in the medical field, and this review discusses their potential in the field of regenerative medicine.

Keywords

Gold nanoparticles (AuNPs), Silver nanoparticles (AgNPs), Iron Oxide nanoparticles (IONPs), Magnetic nanoparticles (MNPs), Regenerative medicine, Biomimetism

1.0: Introduction to Nanoparticles

Nanomaterials are minuscule particles with measurements ranging in the nanoscale, typically 1-100 nm. Nanomaterial synthesis is grounded in multiple disciplines, and nanotechnology as a whole has evolved as a growing domain owing to the strong use of nanoparticles in biomedicine and communications. Nanoscale materials have gained prominence in diverse clinical applications, particularly regenerative medicine and tissue engineering, with variations such as nanoparticles, nanotubes, and nanofibers all becoming extant and tailored to specific applications (Arvejeh, 2024; Sohail, 2025).

Nanoparticles are a version of these nanomaterials and are recognized for their magnetic properties and convenient size. Their minute scale makes them ideal in exerting control over biological functions at a cellular, subcellular, protein, or genetic level (Markides et al., 2012). This controlled nature makes them ideal for various applications, including drug delivery, imaging, and diagnostics.

Regenerative medicine has recently seen a surge in interest in nanoparticles due to limited organ and tissue donors; to combat this limitation, regenerative medicine has advanced the development of scaffolds, which are artificial structures resembling natural materials. Nanoparticles (NPs) gain relevance for their use in surface modification and the release of bioactive agents to strengthen and control these three-dimensional scaffolds (Laird et al., 2021).

NPs can be synthesized by utilizing various materials, with Gold (AuNPs) and Iron Oxide (IONPs) being the most prevalent. IONPs are widely used due to the presence of naturally occurring iron in the human body (in the form of ferritin), indicating their biocompatibility (Markides et al., 2012). Iron-based magnetic NPs are also preferable due to their superparamagnetic nature arising from their nanoscale dimensions, which means they will not be attracted to each other within the human body. IONPs and superparamagnetic NPs in general provide high-resolution Magnetic Resonance Imaging (MRI) contrast and possess impressive penetration power due to their colloidal size (Sun et al., 2008). Similarly, the colloidal size of AuNPs makes them preferable for delivering bioactive agents to diseased sites and cancer cells (Guo et al., 2017).

1.1: Nano-Based Regenerative Therapies

Regenerative medicine is an integrative and multifaceted field centered on the regeneration and repair of cells, organs, or tissues to restore impairment caused by a variety of conditions, including congenital defects, trauma, disease, and aging. It works through research and clinical application, meeting the needs of tissue replacements and drug delivery devices (Sampogna, 2015). Hundreds of regenerative medicine products have been used in clinical development for various diseases, and it combines existing and emerging technological approaches to move beyond traditional replacement therapies (Statista Research Department, 2024). These technological approaches support and induce the natural self-healing pathways of the body with the use of soluble molecules, stem cell therapy, and cell type reprogramming.

As the name implies, regenerative medicine substitutes or regenerates the human body in order to restore natural function, and is regarded as the most dynamically transforming sector in healthcare. Human cells constitute the primary elements of regenerative medicine. These cells may be somatic, adult, or embryo-derived stem cells; there are now versions of the latter that have been reprogrammed from adult cells, so both the natural and the engineered can be conveniently categorized as pluripotent cells (Sen et al., 2025).

Regenerative medicine has evolved tremendously with the advent of nanotechnology, and it has immense promise as a feasible means of restoring function and regenerating diseased tissues and organs. Since cellular function occurs at the nanoscale, nanotechnology can influence and even alter cellular behavior, ultimately enhancing tissue or organ function (Kladko et al., 2021). The conventional approaches of nano-based materials in regenerative therapies can be broadly categorized under nanoparticles, nanofibrous scaffolds, nanotopographically modified scaffolds, targeted delivery of drugs to tissues, and extracellular matrix patterning, as shown in Figure 1. The novel and more integrative approaches deliver a synergy of these traditional methods.

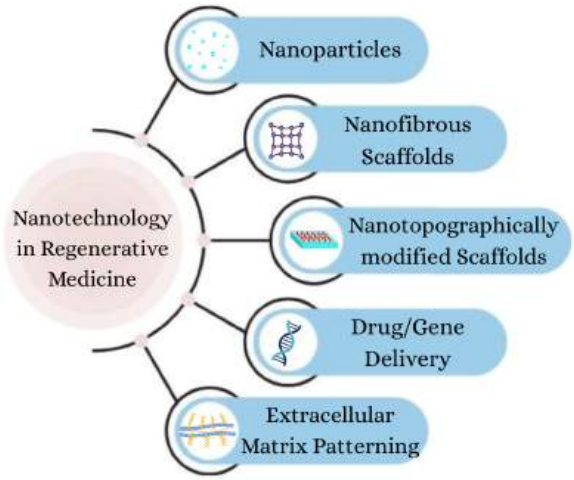


Figure 1: A depiction of the different uses of nanotechnology in regenerative medicine

Nanomaterials have been further progressed into tissue-engineered scaffolds for skin, bone, vasculature, and other tissues. Nanostructures and nanofibrils have also been shown to control the behavior of primary stem cells, including their adhesion, proliferation, growth, and differentiation (Hofman, 2014). To visualize and track cells' independent movements, superparamagnetic iron oxide nanoparticles have been developed, which allow non-invasive monitoring of cells in a living organism (Wang et al., 2025).

Magnetic nanoparticles (MNPs) are notably attractive among the aforementioned emerging approaches due to their unique dimensions and magnetic features. MNPs are generally synthesized with diameters smaller than 20 nm; thus, their magnetizations are prone to fluctuations for biologically workable temperatures. Thus, in the absence of an external magnetic field, their overall magnetization is negligible and randomised to zero (Kolhatkar et al., 2013). Such magnetization directions minimize magnetic interactions between dispersed NPs, thereby stabilizing their diffusion in physiological solutions and facilitating NP pairings with biological agents (Singh and Sahoo, 2014). On the other hand, once these MNPs are exposed to an external magnetic field, they can align with the field direction and achieve magnetic saturation at a greater magnitude that far exceeds that of any known biological entity (Sun et al., 2008). This distinctive property of MNPs offers the dual capability of detecting biological samples containing MNPs as well as their manipulation through an externally applied magnetic field.

MNPs are metal-based, but other variations and compositions exist as well. For instance, carbon-based nanomaterials have unique optical, electrical, and even magnetic properties, but require thorough study of their biocompatibility (Hou et al., 2025). Gas-filled nanobubbles possess notable potential for remote control and ultrasound visualization, but suffer from shorter lifespan in biological systems and technical challenges in functionalization (Jayasankar et al., 2024).

1.2: Challenges in the Clinical Translation of Regenerative Therapies

Significant developments in the healthcare sector have been made in sterilization, organ replacement, and the targeted delivery of drugs to the tissues, all of which have led to an increase in human survival. A contrasting feature has been the evident lifestyle changes, such as a surge in physical activity, underpinning the growing need for tissue replacement therapies.

It is an arduous path to clinical application, and as such, only a few products exist. At this stage, biomanufacturing stem cells or tissues, involving the actual production, packaging, and delivery of a viable product are a few selected parameters which get tested. The emergence of nanotechnology offers a promising perspective in addressing these challenges. The nanoscale of nanomaterials offers sizes comparable to those of biological molecules, thereby enhancing interactions with them and enabling control over various cellular functions (Nag and Delehanty, 2023). Beyond this, nanomaterials with unique optical and magnetic properties are ideal agents for in vivo monitoring cellular behavior following transplantation (Huang et al., 2021).

The biomedical applications of nanoparticles include their use in the delivery of bioactive compounds, integration in three-dimensional scaffolds, and modifying the surface of implanted devices. The combination of these elements is the epitome of nanotechnology in regenerative medicine, and tissue engineering in particular. This article aims to review the synthesis and application of metallic nanoparticles in diagnostic and therapeutic strategies as a revolutionary technique in regenerative medicine.

2.0: Classification and Functional Properties of Nanoparticles in Regenerative Medicine

Nanoparticles can be composed of any material, be it ceramics, metals, polymers, or metal oxides, and are classified accordingly. All types of nanoparticles have their own advantages and unique properties, including biocompatibility, tunable surfaces, and magnetic control, properties which make them highly appreciated in tissue engineering and regenerative medicine as presented in Table 1.

METALLIC/METAL OXIDE NANOPARTICLES			
S. No.	**Type**	**Details**	**Applications & Key Findings**
1.	Silver NPs	**Source**: Silver salt solution reduction **Alternate Source**: Aconitum violaceum extract (Ahmad et al., 2024) **Solvent**: Water; Approx. 10-50nm	Used in scaffolds to treat areas prone to infection; increases blood clotting efficiency (Fathi-Achachelouei et al., 2019)
2.	Gold NPs	**Source**: Turkevich Method (Kimling et al., 2006) **Alternate Source**: Artemisia annua hairy root extract (Bohdanovych et al., 2025) **Solvent**: Water; Approx. 15-40 nm	Bone tissue engineering; Stimulates stem cell differentiation into osteocytes via cellular signaling (Fathi-Achachelouei et al., 2019)
3.	Copper NPs	**Source**: Artemisia annua aqueous extract (Bohdanovych et al., 2025)	Blood vessel formation stimulation; Accelerated collagen deposition

		Solvent: Water; Approx. 35 nm	
4.	Iron Oxide NPs	**Source**: Algal extract (Akhras et al,. 2025) **Solvent**: Water; Approx. 20-50 nm	Magnet-guided scaffold engineering; Scaffold structural reinforcement (Fathi-Achachelouei et al., 2019)
5.	Titanium Dioxide NPs	**Source**: Morus alba extract **Solvent**: Water; Approx. 80-90 nm	Enhances connective tissue survival; Accelerates cell migration in wounds (Su et al., 2025)

Table 1: The different types of metallic/metal-oxide nanoparticles and their applications

2.0.1: Types of Metallic and Metal-Oxide Nanoparticles

Metallic nanoparticles are particularly interesting for their magnetic, electric, and tunable optical properties. In the realm of regenerative medicine, metallic nanoparticles have revolutionized the field by their small-scale actions that enable more precise interactions with biological components (Mendes et al., 2022). NPs are quite interesting in this respect because they regulate certain cellular functions such as proliferation, differentiation, and adhesion of cells (Hofman, 2014). Each NP has its own unique strengths (as illustrated in Figure 2).

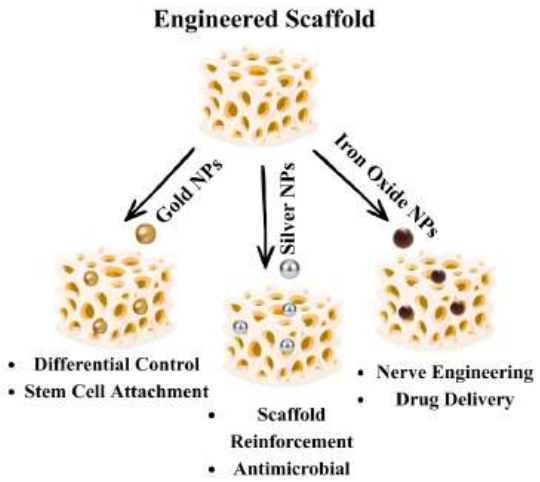


Figure 2: Schematic of metal-based nanoparticle-integrated scaffolds and their applications

2.1: Gold Nanoparticles and Their Applications in Osteogenesis

Gold nanoparticles (AuNPs) are suspended in colloidal solutions and exhibit chemical affinity, allowing them to interact with biological molecules such as peptides and proteins. AuNPs are mainly synthesized through the Turkevich Method by reduction with citrate and ascorbic acid. Developers can then synthesize AuNPs in sizes ranging from 9 nm to 120 nm, initiated by thermal heating or UV irradiation (Kimling et al., 2006). During cytokinesis, modified AuNPs can be directed towards the cell nuclei, where they are able to freeze the cancer cells to stop them from dividing. Some AuNPs can also absorb near-IR light, and when heated by the laser, destroy localised cancer cells without significantly damaging neighbouring tissue.

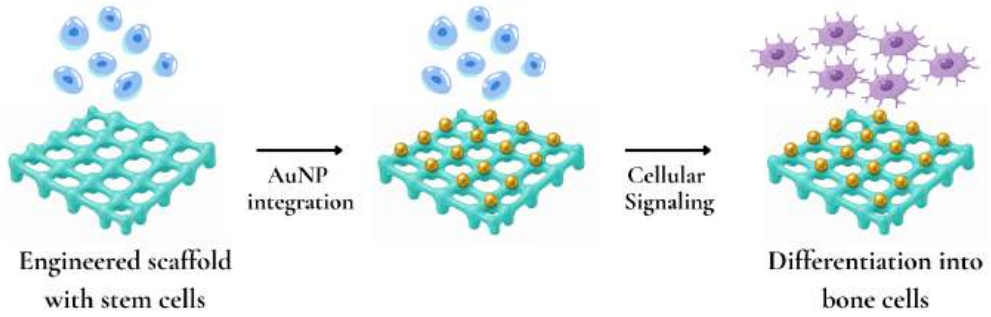


Figure 3: Depiction of AuNP integration in engineered scaffolds to promote stem cell differentiation into bone cells

AuNPs are used to deliver bioactive agents to diseased sites, and when paired with specific antibodies, they target the surface receptors of tumor cells and amplify Raman scattering. This helps in the real-time monitoring and detection of cancer and its relapse. The integration of AuNPs into scaffolds, as shown in Figure 3, also promotes the differentiation of stem cells into bone cells (osteocytes) through cellular signaling, aiding bone regeneration. They can also be laser- or electricity-activated to modulate heart cell pulses and activate muscle tissue, thereby facilitating healing responses. When incorporated into catheter coatings, AuNPs promote stem cell attachment and healthy growth of blood vessel tissue.

2.2: Silver Nanoparticles and Their Use in Scaffold Integration

Silver nanoparticles (AgNPs) are colloidal silver particles suspended in solution and are predominantly valued for their strong antimicrobial properties that help prevent infections in engineered tissues. AgNPs can be synthesized through physical processes (such as evaporation-condensation, laser ablation of bulk material, and gamma or ultrasonic irradiation) as well as chemical approaches (utilizing sodium borohydride or polyol as reducing agents for silver salt solutions). NPs are then formed by nucleation after the reduction of the ions into metallic silver. Bioreduction of silver ions, on the other hand, has emerged as an eco-friendly alternative that utilises natural reducing and stabilizing agents such as microorganisms, bacteria, eukaryotic fungi, or plants to reduce silver ions (Fathi-Achachelouei et al., 2019). AgNPs use various methods to combat both Gram-positive and Gram-negative bacteria; one such method involves attaching to the bacterial cell membrane to disrupt the standard permeability and respiration. Other processes include penetration inside bacteria for direct intracellular damage and the release of silver ions that block the microbial respiratory chain. Figure 4 summarizes these various applications.

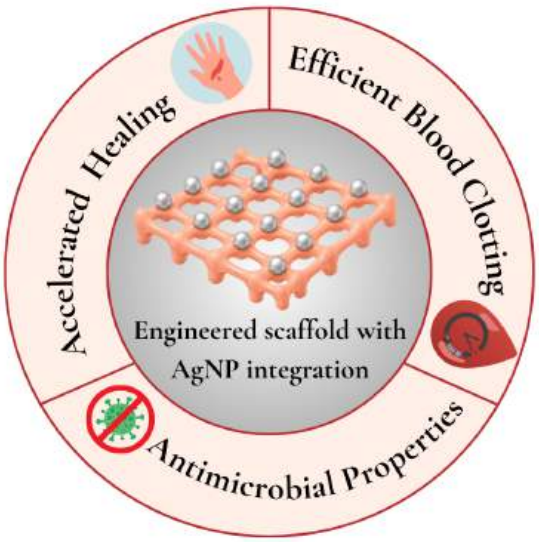


Figure 4: Schematic showing different advantages of AgNP integration in scaffolds

AgNPs are a trusted addition to tissue-engineered scaffolds such as cellulose and gelatin for treating areas highly prone to infection. Studies have noted accelerated healing rates regarding burn wounds being treated with AgNPs compared to standard silver sulfadiazine treatments. It has also been found that AgNPs increase blood-clotting efficiency when combined with chitin scaffolds through interaction with coagulation pathways. They can act as crosslinking agents in decellularized organs to improve stability and have demonstrated toxic effects on breast cancer cells when incorporated into chitosan scaffolds.

NPs with antimicrobial properties, including Silver, Titanium Dioxide, and Zinc Oxide, are often incorporated into engineered tissues to inhibit pathogen growth and create a sterile environment (Hoseinnejad et al., 2017). One such iteration of this is the integration of nanofibrous scaffolds. With fibers ranging between 30 and 130 nm, these scaffolds are designed to mimic the topography of the basement membrane, a thin layer of extracellular matrix that anchors cells to connective tissue (Jayadev and Sherwood, 2017). The characteristics of nanotopography play a crucial role in cell mobility and influence overall cell behavior. For example, aligned nanofibers have been used in nerve regeneration to guide neurite growth, leading to improved propagation when compared to randomly aligned fibers (Kim et al., 2016). Along that same vein, bone scaffolds are frequently reinforced with nanohydroxyapatite-based NPs or carbon nanotubes for maintaining the integrity of the scaffold while providing a biologically similar surface for cell maturation and mineralization (Pei et al., 2019).

NPs are renowned in cartilage regeneration as they circumvent the challenges that make cartilage healing rather difficult, including limited healing abilities and a lack of blood vessels (Jeyaraman et al., 2024). Electrospun nanofibers are used to create an environment to mimic the natural extracellular matrix, facilitating the deposition of specific chemicals necessary for cartilage regeneration (Jiang et al., 2018). In cardiovascular applications, NPs have been used to directly deliver bioactive agents, such as ‘Insulin-like Growth Factor-1’, into myocardial tissue,

reducing scar size and preventing cell death (Kamal et al., 2025). NPs with high oxygen-carrying capacity, such as hemoglobin-based nanofibers, are being considered to enhance the differentiation of stem cells into cardiomyocytes (the cells that make up myocardial tissue) to aid in cardiovascular tissue regeneration (Chaudhury et al., 2014).

2.3: Magnetic Iron Oxide Nanoparticles in Nerve and Tissue Engineering

Iron oxide nanoparticles (IONPs) are a type of magnetic nanoparticle, typically composed of magnetite or maghemite. They are highly valued for their low toxicity and high biocompatibility due to the presence of naturally occurring ferritin within the human body, and are gaining prominence due to their magnetic and optical properties (Nowak-Jerry and Machnicka, 2024). They also aid in the controlled release of bioactive agents, ease biocompatibility concerns, and possess a strong ability to penetrate tissues due to their dimensional advantage. Magnetic IONPs are most commonly synthesized by the co-precipitation method, wherein ferric and ferrous ions are mixed in a highly basic solution (Gupta and Gupta, 2005). Seaweed extracts (Sargassum muticum) have also been used as an eco-friendly biological alternative for synthesising sustainable MNPs (Fathi-Achachelouei et al., 2019).

An important property of IONPs is their superparamagnetic nature, which means that they lose their magnetic properties when the external magnetic field is removed. Certain IONPs with appropriate specifications can circulate in the body for extended periods and be controlled through a localized magnetic field. Therefore, IONPs are universally employed in Magnetic Resonance Imaging (MRI) for the imaging of cancer cells, for tracking stem cells, and monitoring tissues (Li et al., 2016). Superparamagnetic magnetite nanocrystals also have similar applications in cell imaging, as they offer highly sensitive imaging without compromising visibility. IONPs can also cross the blood-brain barrier, making them suitable for delivering biological agents such as peptides and growth factors directly into the brain (Friedrich et al., 2021). IONPs can be coupled with growth factors to stimulate cells, facilitating nerve tissue engineering, thereby helping in tissue engineering. The conjugation of ‘Nerve Growth Factor’ with iron oxide to improve the growth of nerves is one of these examples (Friedrich et al., 2021). A further use for these external magnetic fields is the manipulation of the IONPs to align the collagen fibres in 3D gels, thereby providing structured environments for allowing the natural growth of neurons (Chang et al., 2017).

Tissue engineering is one of the largest applications within regenerative medicine, which involves restoring organ function by combining biocompatible scaffolds with living cells to cultivate functional tissue for implantation (Olson et al., 2011). Tissue-engineered scaffolds act as matrices that support cell growth, and integrating NPs into these scaffolds augments their mechanical strength, chemical properties, and biological advantages. One of the most advanced applications for reinforced scaffolds is the development and use of ferrogels, highly porous scaffolds with MNPs or IONPs embedded within them (Meriem et al., 2022). The magnetic properties of the NPs integrated could allow for externally controlled deformation, where the application of an externally applied magnetic field can alter the volume of the scaffold, triggering

the release of bioactive agents that had been pre-loaded onto the scaffold, such as growth factors, other drugs, and even viable cells (Gao et al., 2015), yielding a high level of control that traditional scaffolds lack.

The incorporation of NPs can also generate scaffolds with active qualities. Drugs, growth factors or other bioactive molecules can be attached to the MNPs, which are then targeted to specific regions of the body through an external magnetic field in a process called magnetic targeting (Mody et al., 2014). This exact control allows small-scale and highly localized accumulation of the biological agents without systemic exposure risks (Yang et al., 2012). Similarly, scaffolds can be reinforced with NPs that are sensitive to environmental changes, such as pH shifts or temperature variations, thereby controlling the release of drugs or growth factors in a manner that models the natural feedback loop during tissue repair.

2.4: MNPs in Stem Cell Applications

NPs are primarily utilized in stem cell research to label, track, and guide multipotent stem cells, as well as control their differentiation into other cell types. The key idea to note here is the difference between multipotent stem cells and the pluripotent ones previously mentioned: pluripotent cells can differentiate into almost any cell type in the body, while multipotent cells are more restricted in that regard (Hasdemir, 2025). Nanofibrils and nanoparticles as a whole have also shown control over differentiation, growth, and adhesion in primary stem cells.

Cell Tracking

Stem cells are labelled with MNPs and monitored within an MRI to track their behavior, movement, and survival in vivo. NPs have critical applications in bioimaging, aiding in the non-invasive and real-time monitoring of the engineered tissues after transplantation or implantation (Srinivasan and Rana, 2024). The main tool used in this specific application is MNPs, since they serve as contrast agents during MRI scans and allow clinicians to track cell growth and distribution as well as the overall efficiency of the treatment (Revia and Zhang, 2016). A prominent obstacle within MRIs is signal dilution, where the signal fades as the cells divide; to combat this, micron-sized particles loaded with smaller IONPs have been developed, providing a stronger and more constant signal (Gao et al., 2015; Bulte et al., 2011).

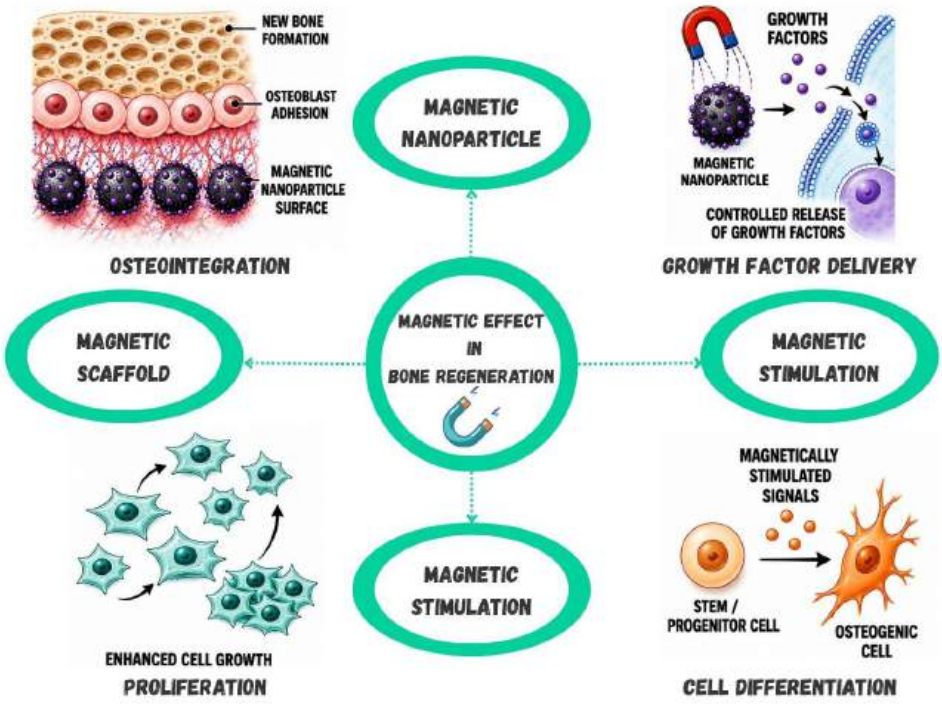


Figure 5: The various applications of magnetism during bone regeneration

Proliferation

As displayed in Figure 5, it has also been recently uncovered that certain NPs actively promote stem cell proliferation. Nanofibrous materials are designed to model the natural extracellular matrix in order to promote the adhesion of various cell types. Nanofibrils and nanoparticles as a whole have also shown control over differentiation, growth, and adhesion in primary stem cells. Superparamagnetic IONPs stimulate the growth of and promote the growth of MSCs, but their concentration must be carefully monitored, since the excessive release of iron can inhibit differentiation of stem cells into certain families (Li et al., 2022; Sharmah et al., 2026). Nanotopographically modifying the surfaces where stem cells are cultured can control differentiation as well, where these modifications, like nanopillars, can act as cues to direct lineage and encourage specific formations (Brammer et al., 2011). One particular application in this regard is magnetofaction: MNPs are associated with genetic vectors, and then the application of an external magnetic field accelerates the sedimentation of these vectors into stem cells (Azadpour et al., 2023). This particular method is useful in the creation of induced pluripotent stem cells and adult multipotent stem cells (MSCs) to express specific proteins to augment regenerative potential (Son et al., 2015).

Differentiation

AuNPs are also rather distinct here, as they utilize cellular signaling to promote stem cell differentiation into bone cells during osteoengineering. AuNPs promote this differentiation by binding to certain cytoplasmic proteins, inducing stress that signals along a protein kinase

pathway. This pathway regulates differentiation, and thus induces osteocytes (bone cells) while inhibiting others (Yi et al., 2010). Further in vitro research suggests that AuNP size as well might play a role in their biological effects, with 20 nm AuNPs being more potent at regulating the activities than larger 40 nm AuNPs. Osteocytes later develop into osteoblasts, and AuNPs increase the viability of these developed primary osteoblasts as well (Zhang et al., 2014).

2.5: Biomimetism and the Protein Corona

In regenerative medicine, biomimetism is the concept of designing materials that can model naturally occurring functions and structures. Manufactured scaffolds must provide the same signals to promote the needed behavior since cells interact with components of the extracellular matrix that are on the nanoscale (Engel et al., 2008). Some instances of this include the creation of nanocomposites to mimic the natural mechanical stiffness of the desired tissues, as well as using nanofibrils to mimic collagen fibers and the basement membrane (Jiang et al., 2015; Frohbergh et al., 2013).

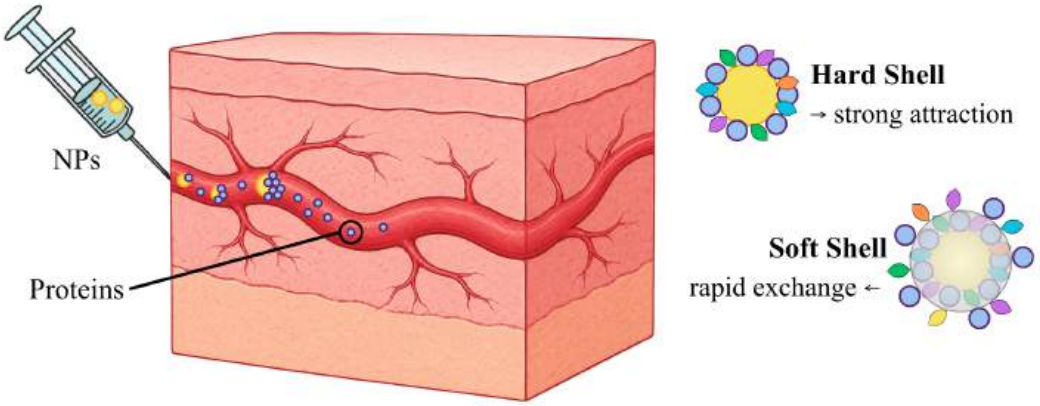


Figure 6: Depiction of protein corona formation in the bloodstream, including the hard inner shell and the soft outer one

Another method of biomimetism is what is known as the protein corona. When NPs enter blood or any other biological fluid, they are immediately coated with proteins, depicted in Figure 6; this coating defines the NPs' biological identity and controls their interactions with nearby cells (Akhter et al., 2021). As shown in Figure 6, proteins first form the "hard" inner shell, where the proteins have a high attraction to the NP surface, and then form the "soft" outer shell, where there is more rapid exchange of molecules. Research has found that even for NPs of a fixed material, the particle size and surface modifications can wholly change the nature of the proteins in the corona and thus possibly the biological impacts (Lundqvist et al., 2008). The composition and dynamics of protein coronas have not yet been fully uncovered, so researchers are now looking to capture the distinct information behind the corona in order to later design NPs that can circumvent immune detection or target certain tissues more efficiently. For example, certain

protein coatings were able to facilitate NP permeation across the blood-brain barrier to mimic natural transport mechanisms (Laabs et al., 2025).

The general excretion of nanoparticles is done through the body's natural waste-clearing systems. Regularly administered NPs are typically metabolized by the body and excreted through feces or urine (Missaoui et al., 2021). A specific instance of this is the application and excretion of Cerium Oxide NPs, which were eliminated primarily through feces and resulted in no reported organ toxicity in certain studies (Hirst et al., 2011). However, in the cases of the newer NPs, exact metabolic pathways and long-term effects of their degradation remain areas that require more advanced research prior to widespread clinical implementations.

3.0: Conclusions and Future Prospects

Nanoparticles have arisen in recent years as a revolutionary mechanism in regenerative medicine by enhancing the administration of therapeutic agents, improving tissue repair, and supporting stem cell therapies. Their incomparable physical and chemical properties include high surface area, integration potential with bioactive molecules, targeted drug delivery, and improved cellular interactions. Nanoparticles have shown promising applications in bone, cartilage, skin, cardiac, and neural tissue regeneration, while also optimizing the application of tissue-engineered scaffolds and minimizing side effects.

Nanoparticles are synthesized from various materials, and the metallic/metal-oxide nanoparticles previously mentioned also possess their own unique properties and advantages. Silver nanoparticles are excellent antimicrobial agents used in infection-prone areas to improve healing rates. Gold nanoparticles, on the other hand, have a variety of uses, such as cancer cell targeting and control over stem cell differentiation via cellular signaling pathways. Magnetic nanoparticles also aid stem cell proliferation, thereby facilitating treatment, and are often used to tag, track, and deliver drugs, cells, and other biological factors. Iron Oxide nanoparticles, a derivative of magnetic nanoparticles, are notable for their enhanced biocompatibility, aiding them in nerve tissue engineering and crossing the blood-brain barrier.

Nanoparticle excretion mainly occurs through natural routes and depends on the biomimetic nature of the nanoparticles delivered. Biomimetism refers to nanoparticles' ability to blend in as part of the natural body, and is owed mostly to the protein corona, a coating of proteins administered to NPs once they enter the bloodstream. Protein coronas hold much potential, but unfortunately, little about their creation and dynamics is known. Notwithstanding these many advances, issues of long-term biocompatibility, toxicity, large-scale production, and clinical adaptation continue to be barriers to broader use.

The future of nanoparticles and their integration in regenerative medicine is promising, driven by advancements in biomaterials and personalized medicine. Further research will be directed toward bringing about the development of biodegradable and biocompatible nanoparticles with minimal harmful effects on the body, the standardization of manufacturing procedures, and the development of a full safety evaluation and regulation for successful clinical translation.

Nanoparticles have immense potential to further the field of regenerative medicine, with novel approaches including enhancing gene editing and delivery with CRISPR therapies and nanoparticle-mediated delivery. The potential use of artificial intelligence and machine learning models to enhance nanoparticle design and biological integration is promising as well. Nanoparticles are also incredibly significant in the future of precision nanomedicine, a future in which nanotechnology is used to develop personalized therapies in the treatment of disease. The dynamics and formation of protein coronas are other applications that need to be studied further, as do the integration of nanoparticles with 3D bioprinting to produce functional organs.

In conclusion, nanoparticles have the potential to revolutionize regenerative medicine by enabling more effective and personal therapies. Continued research, innovation, and rigorous clinical validation will be essential to fully actualize their potential in repairing and regenerating the human body.

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The Dual Role of Dopamine in Brain Health: Insights into Schizophrenia, Parkinson's Disease, and ADHD By Trinity Lio

Abstract

Dopamine is a neurotransmitter with a broad range of roles encompassing neurology, physiology and even psychology. It plays a significant role in disorders like Schizophrenia, Parkinson's and ADHD. In this paper, we reviewed various literature and found that dopamine plays an essential role in healthy brain functioning, but dysregulation, either excessive or depletion, can cause distinct neurological disorders. Excessive dopamine links to schizophrenia, dopamine depletion causes Parkinson's disease, and dopamine dysregulation correlates with ADHD. The crucial role of dopamine dysfunctions is important to understand, as it can explain how one neurotransmitter is capable of producing such respective outcomes and the specific neurological disorders it brings about. Looking ahead, understanding dopamine's dual role can aid future research to improve explanations of these diseases' mechanisms.

Keywords

Dopamine, Schizophrenia, Parkinson's Disease, ADHD, Dopaminergic dysregulation

1. Introduction

Schizophrenia, Parkinson's Disease, and Attention Deficit Hyperactivity Disorder (ADHD) are popular disorders that affect a large percentage of people worldwide, specifically 0.3%, 0.2%, and 8% respectively (World Health Organisation). Schizophrenia is a severe neurological disorder that causes a person to have difficulty with rational thinking and controlling emotions, affecting the person's well-being and quality of life as they tend to struggle with relationships (Stellakis et al., 2024). Parkinson's is a disorder that presents a range of motor and non-motor symptoms that worsen over time (Ramesh & Arachchige, 2023). ADHD is a neurodevelopmental disorder that usually starts in childhood and causes hyperactivity and inattention (Mehler-Wex et al., 2006). Dopamine plays a different role in these three neurological disorders, and this topic is important for understanding the cause of the disease and potential treatments, as these disorders affect people worldwide.

Dopamine is a neurotransmitter produced in the brain, mainly in the substantia nigra, ventral tegmental area, and hypothalamus, which are areas that regulate cognition and emotion. Dopamine plays a crucial role in regulating the reward system and motor control. In 1957, the discovery by Dr. Arvid Carlsson, who concluded dopamine was a neurotransmitter, caused studies on dopamine to begin (Yeragani et al., 2010). Dr. Carlsson's studies not only provided the foundation of dopamine itself but also suggested its relevance to neurological disorders (Yeragani et al., 2010). In addition, dopamine neurons encode the theory of reward prediction error (RPE), which is the difference between expected and received reward (Lerner et al., 2020). Furthermore, dopamine receptors bind with dopamine to regulate the functions of dopamine and are mainly concentrated in the central nervous system (Bhatia et al., 2023). There are five

different dopamine receptors: D1, D2, D3, D4, and D5; they all have different functions and are found in different locations (Bhatia et al., 2023). D1 receptors exist in the largest quantity, mainly concentrated in the striatum and nucleus accumbens, because they aid in regulating neuron development when they are bound to dopamine (Bhatia et al., 2023). In addition, dopamine is also closely related to neurological disorders like Parkinson's, Schizophrenia, and Attention Deficit Hyperactivity Disorder. In Parkinson's, dopamine is deficient due to a degeneration of neurons in the substantia nigra of the ventral midbrain, which causes symptoms like tremor, imbalance, and rigidity (Triarhou, 2013). However, in Schizophrenia, dopamine dysregulation reveals hyperactivity in the mesolimbic pathways from the ventral tegmental area and hypoactivity in mesocortical pathways to the prefrontal cortex (Brisch et al., 2014). This creates positive symptoms such as hallucination and negative symptoms like cognitive deficits (Brisch et al., 2014). On the other hand, in Attention Deficit Hyperactivity Disorder, there is a dysregulation of dopamine signalling in the striatal and cortical areas, leading to symptoms such as hyperactivity and impulsivity (MacDonald et al., 2024).

2. Methodology

This review paper will attempt to explore the role of dopamine concerning its effect on the neurological disorders: Schizophrenia, Parkinson's Disease, and Attention Deficit Hyperactivity Disorder (ADHD).

This review paper followed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) to identify and screen sources to ensure an unbiased and transparent review of existing studies on dopamine's effect on Parkinson's, schizophrenia, and ADHD.

Relevant studies and resources were identified with Google Scholar, PubMed, and PubMed Central. Searches were conducted using combinations of keywords such as 'dopamine', 'schizophrenia', 'adhd', and 'parkinsons'.

The initial search for identification was 'Dopamine AND Schizophrenia OR Attention Deficit Hyperactivity Disorder OR Parkinson's Disease' on PubMed, which yielded 231,673 results. Then, I screened the sources by only using sources after 2021, which yielded 67,613 results. Opinion-based studies, studies that lacked evidence, which were unavailable studies, and duplicates were also excluded.

In total, 21 relevant papers on dopamine and neurological disorders are included in this paper. There are 6 studies relevant to dopamine and Parkinson's, 5 papers relevant to dopamine and schizophrenia, 3 papers relevant to dopamine and ADHD, one paper relevant to dopamine and all three neurological disorders, and 6 papers on dopamine.

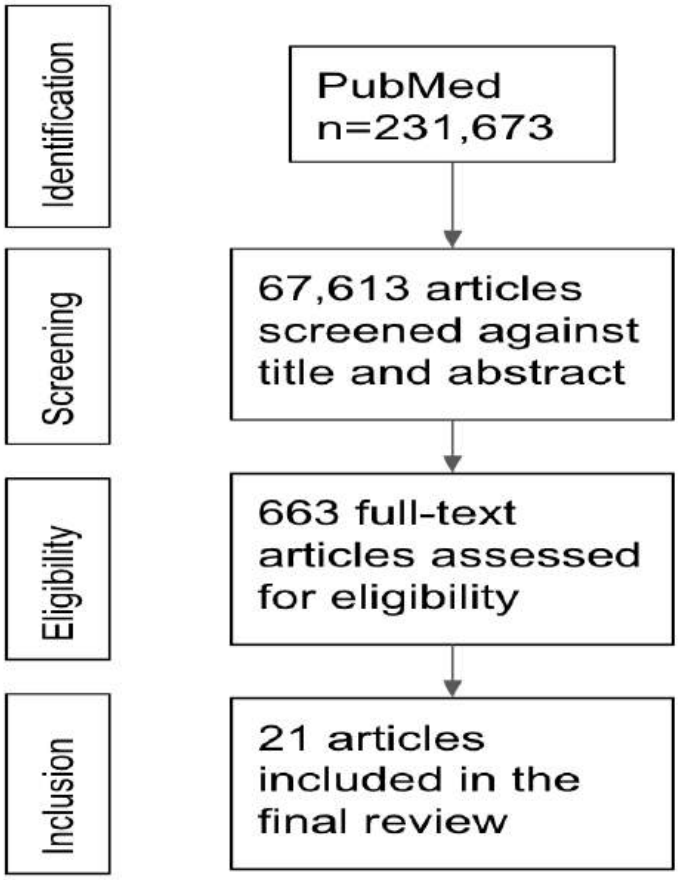


Figure 1: PRISMA diagram

3. Discussion

3.1. Schizophrenia

Schizophrenia is a psychological disorder that hugely impacts a person's behavior, especially causing rational thinking and difficulty with controlling emotions (Stellakis et al.,2024). Although schizophrenia can be caused by multiple factors, like genetics and the environment, one leading cause of schizophrenia is dopamine (Stellakis et al.,2024).

3.1.1 Dopamine hypothesis of schizophrenia

The relevance between dopamine and schizophrenia has been researched for over 70 years (Weinberger, 2022). Originally, the dopamine hypothesis of schizophrenia stated that schizophrenia was caused by excess dopamine (Jauhar et al, 2022). However, the dopamine hypothesis of schizophrenia has evolved as the original one was too simple. Currently, the revised dopamine hypothesis allows areas of dopamine production in the brain to be correlated with symptoms of schizophrenia. It states that hyperactive dopamine transmission in the mesolimbic pathway is linked to positive symptoms, while hypoactive dopamine transmission in

the prefrontal cortex is linked to negative symptoms (Brisch et al., 2014). Positive symptoms of schizophrenia refer to hallucinations and delusions, whereas negative symptoms of schizophrenia refer to avolition and alogia. Furthermore, the modern dopamine hypothesis came around through three different observations (Weinberger, 2022). Firstly, the stimulant drug amphetamine was seen to induce psychosis states, showing that increased dopamine activity can create positive symptoms of schizophrenia. Secondly, antipsychotics affect dopamine metabolism, the process of breaking down and synthesising dopamine. Thirdly, antipsychotic potency is related to dopamine receptor affinity (Weinberger, 2022). In addition, current evidence shows a cortical-striatal imbalance, which is also known as the "DA down- DA up" model, where dopamine is higher in the striatum and lower in the prefrontal cortex (Weinberger, 2022). Evidence suggests that reduced cortical dopamine causes cognitive deficits, whereas increased striatal dopamine causes psychotic symptoms, and a recent PET study was able to provide evidence for this relationship (Weinberger, 2022).

3.1.2 Excess dopamine production

Dopamine is the main neurotransmitter relevant to schizophrenia because the medication used to aid symptoms of schizophrenia, antipsychotic drugs, block the D2 dopamine receptors (Jauhar et al., 2022). In addition, Positron Emission Tomography (PET) imaging studies reveal there are more dopamine receptor occupations in patients with schizophrenia compared to healthy patients (Stellakis et al.,2024). Another study also showed there is high dopamine synthesis capacity in schizophrenia patients with antipsychotic treatment compared to treatment-resistant patients and healthy patients, measured by [^{18}F]-DOPA PET imaging, which explains how excessive dopamine is an explanation for schizophrenia since patients respond to antipsychotic treatment to reduce their dopamine activity (Demjaha et al., 2012). As for those patients who are treatment-resistant, it can suggest their schizophrenia is caused by other factors (Demjaha et al., 2012). One factor that also correlates with dopamine and schizophrenia is the neurotransmitter glutamate (Brisch et al., 2014). Studies show glutamate irregularities can lead to dysregulation in dopamine production, further suggesting glutamate most likely contributes to some symptoms of schizophrenia (Brisch et al., 2014). Another factor is the COMT gene, which affects the rate of dopamine breakdown, influencing cognitive activity (Brisch et al., 2014). Diving deeper into dopamine production, there is evidence to show that there is increased presynaptic dopamine function in the striatum for patients with schizophrenia, specifically increased dopamine synthesis capacity and increased amphetamine stimulated dopamine release (Jauhar et al., 2022). This can be detected in early stages, even before diagnosis of the disorder (Jauhar et al., 2022). Excessive dopamine also causes aberrant salience, where all normal events turn unusually important, forming delusions (Jauhar et al., 2022). This is because dopamine neurons tend to respond to salience, reward, disappointment, and threat; therefore, schizophrenia overcomplicates the importance of these experiences (Weinberger, 2022). This causes a viscous cycle to exist as altered cortical information processing may trigger the wrong dopamine responses, causing abnormal ideas of experience (Weinberger, 2022). A risk factor for

schizophrenia is having a short DRD2 isoform, as reduced expression of the presynaptic dopamine autoreceptor leads to weakening the brain's ability to reject dopamine activity, therefore leading to excess presynaptic dopamine when dopamine neurons are activated (Weinberger, 2022).

3.2. Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative movement disorder that causes symptoms like tremor, postural instability, bradykinesia, and muscle rigidity (Zhou et al., 2023). Parkinson's disease is explained by a loss of dopamine-producing neurons, especially in the substantia nigra, which contributes to the motor symptoms (Ramesh & Arachchige, 2023). The basal ganglia are brain structures that are crucial for movement, cognition, and emotion (Mehler-Wex et al., 2006). Dopamine balances two pathways in the basal ganglia: a direct pathway, which allows movement, and an indirect pathway, which inhibits unwanted movements (Mehler-Wex et al., 2006). In Parkinson's disease, the lack of dopamine in the motor loop causes the direct pathway to be weakened and the indirect pathway to become active, therefore leading to symptoms like tremor and slowness (Mehler-Wex et al., 2006). However, dopamine isn't only important to movement, but also to motivation and memory, further explaining the non-motor symptoms of Parkinson's (Ramesh & Arachchige, 2023).

3.2.1 Dopamine depletion

Dopamine is synthesised from tyrosine and stored in vesicles by VMAT-2 to be released into dopamine receptors (Aguilar et al., 2014). If dopamine isn't stored safely in VMAT-2 and it stays in the cytosol instead, it can oxidize and produce harmful compounds, like DOPAL (Ramesh & Arachchige, 2023). DOPAL (3,4-dihydroxyphenylacetaldehyde) is a toxic dopamine metabolite that causes dopaminergic neuron death (Ramesh & Arachchige, 2023). It damages neurons by binding to proteins, causing mitochondrial dysfunction and ultimately cell death (Ramesh & Arachchige, 2023). Mitochondrial dysfunction leads to dopaminergic neuron death as these neurons require a lot of energy and are sensitive to oxidative stress (Ramesh & Arachchige, 2023). This process is known as the catecholaldehyde hypothesis of Parkinson's disease since DOPAL accumulation is a key factor in dopamine depletion (Ramesh & Arachchige, 2023). Furthermore, a study on Parkinson's disease using a presynaptic dopaminergic PET scan, which targets dopamine synthesis, storage, and transport, was able to identify consistent dopamine loss in patients with Parkinson's disease (Yang et al., 2025).

3.2.2 Dopamine receptor changes

A meta-analysis of D2 receptor imaging studies showed that D2 receptor availability was higher in patients with Parkinson's compared to healthy patients, during the early stages of the disorder (Kaasinen et al., 2021). However, at the advanced stages of Parkinson's, patients have lower D2 receptor availability compared to healthy patients (Kaasinen et al., 2021). This suggested D2 receptors, which work by binding to dopamine receptors, had initial upregulation,

followed by downregulation, most likely caused by the long-term dopaminergic medication use (Nicastro et al., 2021). In addition, the study concluded a clear asymmetry of brain hemispheres in patients with Parkinson's, as D2 receptor binding was 2.8% higher in the side opposite to the patient's most affected body side (Kaasinen et al., 2021). This was also said to help with differentiating between Parkinson's disease and atypical Parkinson's like PSP and MSA (Kaasinen et al., 2021).

3.2.3 Protective and toxic roles of dopamine in Parkinson's disease

In Parkinson's, whilst dopamine depletion causes symptoms, free dopamine can also be toxic, which is why it should be stored in VMAT-2, which is protective as it keeps dopamine away from the cytosol, where free dopamine can turn toxic (Aguilar et al., 2014). However, dopamine can also be broken down by the enzymes MAO and COMT, which produce molecules like hydrogen peroxide that can cause oxidative stress (Aguilar et al., 2014). Free dopamine can be oxidized into three toxic byproducts: reactive oxygen species, dopamine quinones, and DOPAL (Zhou et al., 2023). These metabolites are super toxic and can modify proteins by covalently binding to amino acids to change the function of the protein (Zhou et al., 2023). Moreover, dopamine quinones can boost the toxic alpha-synuclein formation and cause oxidative stress, which explains the cell death in dopaminergic neurons (Aguilar et al., 2014). Aminochrome, a type of dopamine quinone, is a crucial molecule that relates to Parkinson's disease mechanisms as it undergoes redox cycling and continuously generates superoxides and other reactive oxygen species, worsening oxidative damage (Aguilar et al., 2014). But there is a protective enzyme named DT-diaphorase, which reduces aminochrome (Aguilar et al., 2014). Furthermore, neuromelanin is a dark brown pigment in neurons, where there is a lower amount in patients with Parkinson's, as it plays a dual role in dopamine (Aguilar et al., 2014). It is protective by storing toxic dopamine products and metals, but it can be toxic if there is an iron overload or neuron death, increasing oxidative stress (Aguilar et al., 2014).

3.3. Attention Deficit Hyperactivity Disorder (ADHD)

Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder that is largely caused by irregularities and disruptions of dopamine (Pagán et al., 2023). Dopamine has a crucial role in ADHD because dopamine affects attention, motor control, cognitive abilities, and emotion regulation, which are all factors of ADHD (Pagán et al., 2023).

3.3.1 Dopamine dysregulation

Dopamine irregularities are a part of a larger dysfunction in frontostriatal, frontal parietal, and reward-related circuits (Pagán et al., 2023). Dopamine dysregulation leads to a lack of motivation because the altered reward processing makes it hard to attend to tasks that aren't rewarding (Pagán et al., 2023). There are dopamine irregularities in the motor and limbic loops, which cause the hyperactivity and inattention in ADHD (Mehler-Wex et al., 2006). The motor loop is also less active, making it harder to stop unwanted movements (Mehler-Wex et al., 2006).

Furthermore, a deficiency in the dopamine transporter causes a decline in dopaminergic response strength, which causes poor regulation of attention (Pagán et al., 2023). Changes in the dopamine receptor response are also said to contribute to delayed brain development and maturation of ADHD (Pagán et al., 2023). Consequently, dopamine signalling of the importance of events is hugely relevant to the impaired processing of reward anticipation and reward salience in ADHD (Pagán et al., 2023). Therefore, the main treatments for ADHD are psychostimulants, which are effective as they increase dopamine by targeting dopamine transporters (Mehler-Wex et al., 2006). Methylphenidate and amphetamine-based medications are also efficient because they increase dopaminergic signalling and aid with symptoms of ADHD (Pagán et al., 2023; Dum et al., 2022).

3.3.2 Dopamine receptors and their contribution to ADHD

Irregularities in specific dopamine receptors are crucial to understand, as each dopamine receptor contributes to different aspects of symptoms of ADHD (Dum et al., 2022). The strongest dopamine receptors relevant to ADHD are D1, D2, D3, and D4 (Dum et al., 2022). Firstly, D1 receptors are crucial to attention and memory regulation in the prefrontal cortex and striatum (Dum et al., 2022). Human studies link D1 receptor polymorphisms to inattentive symptoms, and mice knockout studies further show hyperactivity and poor task performance, mirroring ADHD symptoms (Dum et al., 2022). Secondly, D2 receptors are linked to planning, working memory, locomotion, impulsivity, and reward (Dum et al., 2022). Animal models and human studies show irregularities in dopamine are associated with impulsive behavior and exaggerated reward behavior (Dum et al., 2022). Thirdly, D3 receptors are related to reward processing, motivation, emotion regulation, and prefrontal neurocognition (Dum et al., 2022). D3 receptors are especially relevant to hyperactivity, impulsivity, and traits seen in adult ADHD (Dum et al., 2022). Lastly, D4 receptors are the most important dopamine receptor gene seen in ADHD; they regulate corticostriatal circuits and synaptic signalling (Dum et al., 2022). Specifically, the 7R allele of the D4 receptors, which is known as an ADHD risk factor, is linked to cortical development differences and behavioral factors in ADHD (Dum et al., 2022).

4.0 Discussion

4.1. Dopamine as a Double-Edged Neurotransmitter

The main finding is that dopamine is essential for normal brain function but becomes harmful when dysregulated. Both excessive or insufficient dopamine levels can lead to neurological dysfunction, further concluding how dopamine levels should be maintained at an optimal physiological range for healthy brain activity.

Dopamine's double edge nature can be seen throughout the three neurological disorders examined: Schizophrenia, Parkinson's disease, and ADHD. In schizophrenia, excess dopamine in the mesolimbic pathway is hugely linked to positive symptoms, like hallucinations and delusions. Contrastingly, Parkinson's disease is explained by a loss of dopamine-producing

neurons and a severe dopamine depletion, causing the direct pathway to be weakened and the indirect pathway to become active, contributing to motor symptoms. On the other hand, ADHD shows dopamine dysregulation, rather than absolute excess or deficiency, affecting the different symptoms of ADHD, like impulsivity, attention, and motivation. These key findings can suggest the fact that one neurotransmitter can cause different clinical outcomes depending on the extent of its production and location.

4.2. Comparing Dopamine Dysfunction Across Disorders

All three disorders feature altered dopaminergic signalling where specific brain circuits are affected. These changes in dopamine affect cognition, behaviour, movement and motivation. In schizophrenia, the revised dopamine hypothesis discusses hyperactive dopamine transmission in the mesolimbic pathway being linked to positive symptoms. Whereas, hypoactive dopamine transmission in the prefrontal cortex is correlated to negative symptoms. This shows the simultaneous 'hyper' and 'hypo' states produce different symptoms.

In Parkinson's disease, there is severe dopamine depletion caused by the progressive degeneration of dopaminergic neurons in the substantia nigra. This leads to dominant motor symptoms but cognitive symptoms may also emerge over time.

In ADHD, dopamine dysregulations are more specific, particularly with impaired dopamine signalling in the frontostriatal and reward-related circuits. This causes reduced reward sensitivity, difficulties handling motivation, and executive dysfunction, without excessive or depletion of dopamine like the other two disorders.

Therefore, the same neurotransmitter, dopamine, produces different disorders depending on where and how dopamine is disrupted.

4.3. Clinical Implications

Understanding dopamine has significantly improved and enhanced the treatment of these neurological disorders. In schizophrenia, D2 receptor antagonists effectively reduce positive symptoms by reducing dopamine transmission, even though negative symptoms still remain difficult to treat. In Parkinson's, levodopa and other treatments restore dopamine levels and aid with motor symptoms. However, these long-term treatments may lead to complications, like dyskinesia and motor fluctuations, and neuroprotective therapies still need more research. In ADHD, stimulants, like methylphenidate and amphetamines, help improve dopamine transmission and are highly effective in improving attention and reducing impulsivity. However, this varies between individuals.

Nevertheless, current therapies only modify dopamine signalling and rarely address these underlying disease mechanisms. Dopamine doesn't act independently, but it is a neurotransmitter that interacts with other neurotransmitter systems, like glutamate. Therefore, future research and models should integrate different neurotransmitter systems and molecular pathways, as such advances would hugely improve the precision and quality of these treatments across these disorders.

5.0. Limitations

There are a few limitations that are considered in this review paper. Firstly, there was a limited number of relevant papers, and only recent papers were reviewed. Secondly, other neurotransmitters can be equally as influential as dopamine in these neurological disorders. Thirdly, different kinds of studies, like PET, animal models and clinical studies, can't be directly compared with one another.

6.0 Conclusion

This review paper has shown dopamine as a neurotransmitter having a dual role. Whilst it is essential for healthy brain function and everyday life, it can produce neurological disorders when its signalling becomes excessive, depleted, or dysregulated. Excessive dopamine brings about schizophrenia, dopamine depletion drives Parkinson’s disease, and dopamine dysregulation contributes to ADHD.

Understanding these different dopamine dysfunctions is important as it explains how and why the same neurotransmitter is able to produce different outcomes and why current treatments, despite being effective in alleviating symptoms, are still lacking in explaining the underlying disease mechanisms. Ultimately, recognising dopamine as double-edged can guide future research to more precise approaches to gain more insight into this neurotransmitter. Future studies should take into account the roles of other neurotransmitters in addition to dopamine as it is not the only factor in the disorders studied.

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Health Inequality: How Medical Devices Are Widening the Gap By Chaeyoon Cho

Abstract

A device that can track heart rate, sleep, and blood oxygen may seem like a simple tool for better health, but its value depends on who can access it and how well it works for different users. This paper identifies four major areas of concern in the use of wearable health technology: unequal access, low adoption among high-risk patients, potential sensor inaccuracies across skin tones, and structural barriers such as cost, digital literacy, and limited clinical integration. To examine these issues, a cross-sectional, mixed-methods survey was completed by 15 adults living in South Korea. Together, the literature and survey findings suggest that wearable technology may reinforce health disparities unless it becomes more affordable, accessible, clinically connected, and accurate for diverse users.

Introduction

Healthcare technology is often seen as a solution to medical problems because it can improve treatment, track symptoms, and help detect diseases earlier. Devices such as smartwatches, smart rings, and other advanced medical tools are becoming increasingly common. Yet the benefits do not reach everyone equally. Wearable technology is used most often by people who are already younger, wealthier, and healthier, while older adults, low-income individuals, and people with serious health conditions are less likely to access or benefit from it. Some devices may also produce less accurate results for people with darker skin tones. These patterns raise an important question: does wearable health technology close health gaps, or does it widen them along lines of income, race, and age?

Health inequality refers to systemic differences in health outcomes across social groups, often resulting from unequal access to resources, medical care, and health technologies. Medical wearables are consumer/clinical devices that can track physiological data such as heart rate, sleep, and blood oxygen. Although these devices are designed to support prevention and early detection, uneven access and performance mean they also widen the gap between people who can benefit from health technology and those who cannot, a gap this paper refers to as the digital health divide.

Four patterns illustrate this divide. First, adoption is skewed toward the people who need wearable technology least: higher median household income is associated with greater wearable use, while older age and existing conditions such as heart failure are associated with lower use (Venn et al., 2024). Cost remains a major barrier to closing this gap. In one large health system survey, “95.3% of nonusers reported that they would use a wearable device if it were provided at no cost”, showing that financial barriers strongly affect access (Venn et al., 2024). National survey data confirm that ownership is far more common among younger, higher-income, and more highly educated adults than among older or lower-income groups (Nagappan et al., 2024).

Second, accuracy is a concern, as some sensors may produce less accurate results for darker skin tones. Many wearable devices rely on photoplethysmography (PPG), which estimates

heart rate from light reflected off the skin. Because melanin absorbs more light, PPG sensors may produce less reliable readings for people with darker skin tones (Asif et al., 2025), meaning that inequality can persist even after a person gains access to a device.

Third, the patients who could benefit most from continuous monitoring are not the ones driving adoption. Among wearable owners in one national sample, nearly one-third rated their own health as "excellent," more than twice the rate reported by nonowners (Nagappan et al., 2024), a pattern suggesting that the healthiest individuals, rather than those with the greatest medical need, are most likely to benefit. As a result, inequality can continue even after a person gains access to the device. Together, they show that wearable technology may not always be designed or distributed to meet medical needs.

Fourth, wearables remain poorly integrated into clinical care. Most owners purchase their devices themselves rather than receive them through a clinician. It is said that only 8.1% of owners in the Nagappan et al. (2024) sample received a device from a doctor, and in the Venn et al. (2024) sample, only 24.8% of owners had shared their device with a physician, while just 7.8% reported that a doctor had recommended a wearable for medical reasons.

Together, these four patterns show that wearable technology is often marketed as a healthcare solution, but its actual distribution runs opposite to what equitable healthcare would require. Adoption is concentrated among the healthiest, wealthiest, and most digitally engaged users, while the devices themselves may work less reliably for marginalized groups and remain disconnected from the clinical systems that could translate their data into care. These four patterns have been documented largely in large U.S. datasets. This study reports an original cross-sectional survey of 15 adults in South Korea to examine whether similar patterns appear in a smaller, non-U.S. sample.

Methods

This study used a cross-sectional, mixed-methods survey to investigate how access to and experience with wearable health technology differ across different groups. The survey collected data through multiple-choice and rating-scale questions, while open-ended free-response questions allowed participants to explain their experiences in greater detail. It was designed to test patterns already established in larger studies of wearable use, digital access, and sensor accuracy – including lower wearable use among older adults and lower-income groups, and concerns about the accuracy of light-based sensors for people with darker skin tones. Because the participants completed the survey only once, the study can identify connections between variables but cannot determine direct cause-and-effect relationships.

Adults aged 18 and older were eligible to participate regardless of whether they owned a wearable device or had a diagnosed medical condition. Including both users and non-users was necessary because the study focuses not only on experiences with wearable technology but also on the reasons some people do not use it. Recruitment aimed to include participants across a range of ages, incomes, health-risk levels, and skin tones, with particular attention to reaching

older adults, lower-income individuals, people with chronic health conditions, and people with darker skin tones.

The survey was first shared with members of the researcher's family, including parents, grandparents, and cousins, who then distributed it to friends and other people in their networks. This helped the survey reach adults of different ages, but because it relied on personal connections, the participants likely shared overlapping cultural, social, or economic backgrounds. Therefore, the sample may underrepresent people with limited digital access or serious chronic illnesses.

Data were collected via Google Forms, with participants completing either the English or Korean version of the survey, based on their preference. The survey asked about age, gender, race or ethnicity, skin tone, household income, education, insurance status, health conditions, digital access, wearable ownership, device use, perceived accuracy, and barriers to use. Skin tone was measured separately from race using the ten-point Monk Skin Tone Scale. Participants who did not own a wearable device were automatically routed past questions about usage and perceived accuracy to the barriers section. The open-ended questions were optional, since one asked participants to describe an inaccurate or confusing wearable reading, which nonusers could not answer. ZIP code was also optional because it can be semi-identifying and therefore required more careful handling.

Responses were summarized using counts, percentages, and simple comparisons. Wearable ownership and use were compared between higher-risk and lower-risk participants to examine whether those with greater medical needs were less likely to use these devices. Barriers to wearable use, such as cost, lack of interest, limited digital access, and low trust, were compared across income and technology-access groups. Responses about device accuracy were also reviewed by skin tone and device type. Open-ended responses were examined using thematic coding, with recurring ideas grouped into themes such as affordability, inaccurate readings, trust, accessibility, and unequal healthcare benefits.

Participants read an informed-consent statement before beginning the survey. Participation was voluntary, and responses were collected anonymously without names or email addresses. Health information, income, race, skin tone, and ZIP code were treated as sensitive data, and a "prefer not to say" option was included for relevant questions, allowing participants to decline to share information they were uncomfortable providing.

This study has several limitations. The small sample size limits the strength of subgroup comparisons and the generalizability of the findings. Because all respondents lived in South Korea and were recruited through family and social networks, the sample is not representative of the broader population. Online recruitment may also underrepresent people with limited internet access or low digital literacy, even though these groups are central to the study. Self-reported responses may not perfectly reflect actual performance, especially because users may not notice minor errors, and the visual skin-tone scale is less precise than clinical measurements. Finally, because the survey was cross-sectional, it can identify patterns and associations but cannot determine causation.

Results

15 adults aged 18 or older completed the survey, all of whom resided in South Korea. The sample spanned a range of age groups and income levels, allowing the survey to examine whether financial background relates to wearable ownership or access. Because the sample size was small, however, the results are best read as general patterns rather than strong statistical conclusions.

Most participants did not report major chronic health conditions. One participant reported having both hypertension and heart disease or heart failure. This participant was also the only respondent who reported being monitored by a doctor for a chronic condition. One participant reported being 65 years old or older. Because the sample included only a small number of older adults and high-risk participants, the study had limited ability to represent the groups most likely to benefit from continuous health monitoring.

The survey also examined participants' access to technology and comfort with digital tools. Responses varied in reported reliability of home internet and comfort using health-tracking apps or new technology generally, with no clear relationship between age and digital comfort in this sample. Overall, digital access did not appear to be the strongest barrier reported by participants. Instead, the main barriers were cost and the belief that wearable devices were not medically necessary.

Of the 15 respondents, five (33%) reported currently owning or using a wearable health device, meaning that most participants did not. Among wearable owners, participants acquired their devices in different ways. Some purchased their own, while others received one as a gift, and reported that costs varied, indicating that wearable ownership can require different levels of financial access. Among nonowners, the most common reason was that they did not see a need for one. This suggests that nonuse was related not only to cost but also to participants' belief that the device was necessary for their health.

The survey also asked whether doctors influenced participants' use of wearable devices. In this sample, there was little to no connection between wearable use and medical guidance. Participants rarely reported that a doctor had recommended a wearable device, and wearable data were rarely shared with a doctor or other healthcare provider. This suggests that, for this group, wearable devices were used more as personal consumer technology than as tools integrated into medical care.

Discussion

The survey results partially support the argument that wearable health technology can widen health inequality, although the evidence from this sample is limited. Several findings align with the broader concern that wearable devices are not equally accessible, are not consistently connected to medical care, and do not necessarily reach the people who could benefit most. At the same time, the sample did not include many older adults or high-risk participants, so the results cannot fully confirm every part of the argument.

The strongest support came from the access-related findings. Only five of 15 respondents reported owning or using a wearable device, and among nonowners, cost and a lack of perceived medical need were the main barriers, evidence that wearable technology is not automatically available or meaningful to everyone. The sample lacked sufficient income diversity; however, to identify a clear income-based pattern, a larger sample with more low-income participants would be needed to better measure the relationship between income and ownership.

The findings also suggest that wearable devices were not strongly integrated into medical care in this sample. Participants rarely reported that a doctor recommended a wearable device, and wearable data was rarely shared with a healthcare provider. This matters because devices marketed as health tools may have limited medical value if their data never reaches a clinician. Because only five participants owned wearables, though, this finding should be read as suggestive rather than conclusive.

The survey was less able to evaluate whether high-risk patients use wearables less than others. Only one participant reported both hypertension and heart disease or heart failure, and this was the same participant who was monitored by a doctor for a chronic condition: only one participant was also 65 years old or older. This limited representation became a limitation of the study itself. The survey did not reach enough of the groups most central to the research question. Future work would need to intentionally recruit more older adults and people with chronic illness to test whether wearable technology reaches the patients with the greatest medical need.

The accuracy issue was also difficult to test with this sample. The survey included questions about trust, perceived accuracy, and awareness that some wearable sensors may be less accurate for darker skin tones. However, the small number of participants and limited skin-tone variation made it difficult to detect a clear relationship. This part of the argument is better supported by existing research (Asif et al., 2025) than by the current survey data; a larger, more skin-tone-diverse sample would be needed to test it directly.

Overall, the survey results provide partial support for the idea that wearable health technology may reinforce health disparities. The clearest findings were limited ownership, cost- and perceived-need barriers, and a weak connection between wearable devices and doctors. These results suggest that wearable technology does not automatically improve healthcare access simply by existing. However, the study's small, nonrandom sample limits the strength of the conclusions, especially for high-risk patients and darker-skinned users. The findings should therefore be treated as exploratory, pointing to patterns that would need to be tested in a larger, more diverse study rather than settled conclusions.

Conclusion

Wearable health technology is often described as a major advance in modern healthcare, yet evidence shows that its benefits are not always distributed equally. Existing research and the survey results reported here both suggest that wearable devices are most accessible to people who already have financial, technological, and health advantages. While these devices can help users track symptoms, monitor health patterns, and detect potential problems earlier, they may

also exclude people who face cost barriers, have limited digital access, or need stronger medical support.

These findings carry important implications for both wearable design and healthcare policy. Wearable devices should be tested across a wider range of populations so they work accurately for people of different ages, income levels, health conditions, and skin tones. Policies should focus on making helpful devices more affordable and better connected to clinical care, particularly for patients who may benefit from continuous monitoring. If wearable data is never connected to a doctor or healthcare system, the device may remain a personal consumer product rather than a meaningful medical tool.

A follow-up study would need a larger and more diverse sample to test these patterns more clearly. Future research should include more low-income participants, older adults, high-risk patients, and people across a wider range of skin tones. This would make it possible to better examine whether income affects ownership, whether darker skin tones are associated with greater accuracy concerns, and whether high-risk patients are actually receiving the tools they need. Wearable technology should not be viewed as an automatic solution to healthcare inequality. Medical devices can only reduce health gaps if they are affordable, easy to use, clinically connected, and tested across diverse populations. Without these changes, wearable devices may continue to benefit healthier and wealthier users first, while the people who need continuous health monitoring the most receive fewer benefits. Healthcare innovation should be measured not only by how advanced it is, but also by who can actually access and benefit from it.

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Student Awareness and Misconceptions of Creatine Monohydrate Use in High School Populations By Adam Gharibnavaz and Eviee Zhang

Abstract

Creatine monohydrate (CM) is a highly controversial supplement used in adolescent environments because it is rarely introduced to teenagers through a single clear message. This study examined how high school students judge CM when their information comes from a mix of peer discussion, fitness content, family concerns, and safety warnings. Using a school-based questionnaire with 69 valid responses, the research explored the difference between having heard of CM and being able to explain it accurately. Findings indicated that students recognized CM more through its reputation than its biology. Strength, muscle growth, and athletic performance appeared more clearly in student responses than ATP-related energy support. Safety information and scientific evidence had the strongest influence on decision-making, while negative views were mainly connected to health concerns and steroid confusion. Overall, the study presents CM as an information problem as much as a nutrition topic. Better supplement education can help students replace assumptions with clearer judgment in the future.

Introduction

Creatine monohydrate (CM) is a thoroughly researched dietary supplement that is used worldwide, yet its scientific function is heavily simplified and distorted in adolescent fitness culture. In the United States, creatine consumption has been reported to exceed 4 million kilograms per year, highlighting its consistent use among athletes, bodybuilders, and recreational exercisers (Desai et al.). This popularity has made CM highly visible in gyms and online fitness content, at the cost of being poorly understood. Scientifically, CM is not an anabolic steroid or an artificial shortcut for growing muscle (Antonio et al.). It is a naturally occurring nitrogen-containing compound active in cellular energy metabolism, specifically during brief periods of high energy demand (Wyss and Kaddurah-Daouk).

Modern controversy surrounding CM originates from the distance between its biochemical role and its public reputation. Sports nutrition research links CM to improvements in repeated-sprint ability, resistance-training output, lean body mass, and recovery when supplementation is incorporated into a structured training program (Wax et al.; Desai et al.; Wu et al.). Concurrently, public discussion frequently emphasizes kidney stress, dehydration, cramping, hair loss, water retention, and comparisons to anabolic steroids (Antonio et al.; Mayo Clinic). General caution is appropriate in certain cases where individuals have pre-existing medical conditions or when dosing practices are unsupervised (Cleveland Clinic). However, much of the stigma surrounding CM comes from misunderstanding normal creatine metabolism or confusing CM with substances that function through hormonal pathways (Antonio et al.). This study examines the persisting gap between scientific evidence and student comprehension through a cross-sectional survey of high school students, focusing on how they hear about, interpret, and judge CM.

Literature Review

Physiological Mechanism and ATP Regeneration

Creatine's main physiological value stems from its role in the phosphocreatine (PCr) energy system. Before performing its function in this system, creatine is synthesized endogenously from the amino acids arginine, glycine, and methionine through a two-step inter-organ pathway. In the first of the two steps, arginine:glycine amidinotransferase, called AGAT, catalyzes the reaction between arginine and glycine to form guanidinoacetate and ornithine, mainly in the kidney. Then, in the second step, guanidinoacetate is transported to the liver, where guanidinoacetate N-methyltransferase, also known as GAMT, uses S-adenosylmethionine to methylate guanidinoacetate into creatine. This process is highly metabolically demanding, as creatine synthesis can consume 40% to 70% of the available methyl groups from S-adenosylmethionine (Wyss and Kaddurah-Daouk; Allen). The body maintains its creatine supply using this endogenous process as well as dietary intake, with animal-based foods like fish and meat serving as common dietary sources (Wyss and Kaddurah-Daouk; Wax et al.). After synthesis or ingestion, creatine enters circulation and is transported into tissues with high energy demand. About 95% of the body's creatine is stored in skeletal muscle, with smaller amounts present in tissues such as the brain, heart, and testes (Kreider et al.; Wax et al.). Inside skeletal muscle, creatine exists primarily as PCr and free creatine, with approximately two-thirds of intramuscular creatine stored as PCr and the remaining portion as free creatine (Kreider et al.).

The PCr system functions as a swift ATP-buffering mechanism. During intense muscle contraction, ATP is broken down into ADP and inorganic phosphate. When ATP demand rises faster than aerobic metabolism can respond, an enzyme called creatine kinase catalyzes the transfer of a phosphate group from PCr to ADP, regenerating ATP. This reaction is reversible, ensuring that ATP can later re-phosphorylate creatine and rebuild the PCr pool during recovery (Wyss and Kaddurah-Daouk; Allen). This explains why CM is most relevant to short-duration, intense activity. The supplement does not create endurance energy in the same way aerobic metabolism does. Instead, it supports the first seconds of intense effort, when rapid PCr depletion limits the muscle's ability to replenish ATP quickly (Wax et al.).

Supplementation is used because normal muscle creatine stores are not always completely saturated. Exogenous CM can increase intramuscular creatine and PCr stores by roughly 20% to 40%, depending on baseline levels (Kreider et al.; Wax et al.). This increase does not create a new energy system, but rather it expands the capacity of an existing one. This distinction is important for adolescent understanding, as CM is often described socially as a direct muscle-building substance, even though its primary role is to support energy buffering.

Performance and Training Adaptation

Performance benefits associated with CM are most evident in activities that require short bursts of force. Reviews have reported noteworthy improvements in maximal power, strength, anaerobic capacity, and repeated sprint work after creatine supplementation (Wax et al.). These

effects are not equally relevant to every form of exercise. CM has less direct value for long-duration endurance exercise than for resistance training, sprinting, and power-based sport tasks, where rapid PCr resynthesis affects later performance (Wax et al.).

Body composition research from recent years gives such evidence more specificity. In a review and meta-analysis on adults younger than 50, researchers screened 1,694 records, assessed 67 full-text articles, and included 12 studies in the final meta-analysis. Compared with resistance training by itself, creatine combined with resistance training was found to increase lean body mass by 1.14 kg and reduce fat percentage and fat mass by 0.88% and 0.73 kg, respectively (Desai et al.). These findings support the idea that CM can augment resistance-training adaptation, but they also clarify that CM has its strongest body-composition effects within a training context. Resistance training remains the main stimulus for hypertrophy, and CM appears to improve the physiological conditions that permit training adaptations to accumulate (Desai et al.).

A scoping review of randomized clinical trials from 2012 to 2021 further narrows where this effect appears strongest. CM was considered most effective for muscle growth in healthy young individuals participating in adequate training (Wu et al.). Some trials reported increases in muscle strength after exercises such as bench press, leg press, and shoulder press, and another trial found lean soft tissue increases of 7.1% in the upper limbs, 3.2% in the lower limbs, and 2.1% in the trunk (Wu et al.). However, the same review found weaker results in clinical populations, where some studies reported no significant changes in muscle function, aerobic capacity, fatigue, or quality of life (Wu et al.). All in all, CM has the strongest support in healthy active populations, with less consistent effects in disease-related, older, and immobilized groups.

CM's connection to muscle growth is more indirect than public discussion implies. Its effects may come from greater PCr availability, faster ATP resynthesis, increased training capacity, cell hydration, and possible cellular changes related to muscle adaptation (Desai et al.). However, acute supplementation research cautions against describing CM as a direct protein-synthesis trigger. In one controlled study, healthy young men and women consumed 20 g/day for five days followed by 5 g/day for three to four days. CM increased total muscle creatine and produced a slight increase in PCr, but it did not increase mixed-muscle protein synthesis or total body mass during the short intervention. In male participants, it reduced leucine oxidation and plasma leucine rate of appearance, indicating a possible anticatabolic effect instead of direct muscle-building activity (Parise et al.).

Dosing, Water Retention, and Safety

When CM dosing and side effects are discussed without proper context, major misunderstandings about the supplement can become normalized. A common loading protocol, a short higher-dose period used to saturate muscle stores faster, uses roughly 0.3 g/kg/day for five to seven days, followed by a maintenance dose of 0.03 g/kg/day for about four to six weeks (Gutiérrez-Hellín et al.). However, loading is not required for CM intake. Lower daily doses, such as 3 g/day for about a month, can still increase intramuscular creatine and PCr stores over

time, although this approach takes longer (Gutiérrez-Hellín et al.). After supplementation stops, intramuscular creatine levels may take five to eight weeks to return to baseline (Gutiérrez-Hellín et al.). This scientific context contrasts with informal gym advice, which may emphasize rapid results without explaining dose, timing, or saturation.

Safety concerns require careful separation between appropriate caution and unsupported fear. Public-facing medical sources describe CM as safe for many healthy users but recommend speaking with a healthcare provider before use for individuals with kidney disease, liver disease, diabetes, pregnancy, breastfeeding, medication use, or other medical considerations (Cleveland Clinic; Mayo Clinic). Reported side effects can include gastrointestinal discomfort, nausea, diarrhea, dizziness, and weight gain from water retention, with some effects more likely when CM is taken in high doses over a short period of time (Cleveland Clinic; Gutiérrez-Hellín et al.). These side effects help explain why students may think CM is dangerous, but they do not prove that the supplement is inherently harmful. Common public concerns involve water retention, dehydration, cramping, kidney function, hair loss, and comparison to anabolic steroids (Antonio et al.; Mayo Clinic).

Water retention is one of the clearest examples of a real physiological effect being socially misinterpreted. CM is hygroscopic, meaning the compound itself tends to attract water, so increased CM uptake into muscle cells can raise intracellular water content. Short-term CM supplementation, mainly during a loading phase, can increase body mass by about 1 to 3 kg after five to seven days (Gutiérrez-Hellín et al.). In research, this is described as intracellular water retention or cellular hydration. In public discourse, the same effect may be described as bloating, fake muscle, or unhealthy weight gain. These labels can make early weight change seem more harmful than what current evidence actually suggests. Dehydration and cramping concerns come from a related misunderstanding. If creatine pulls water into muscle cells, some people may assume that it must dehydrate the rest of the body. However, controlled research has not consistently identified dehydration or muscle cramping as typical side effects of CM supplementation when used appropriately by healthy individuals (Antonio et al.; Allen).

Kidney-related concerns are another major example of safety confusion. Creatinine is a breakdown product of creatine metabolism and is commonly used as a marker in kidney function testing. CM supplementation can increase creatinine levels, but this does not automatically indicate kidney damage. It may reflect increased creatine turnover instead of impaired renal function (Antonio et al.). Some sources also explain that creatinine-based kidney tests can be misread in supplement users if clinicians do not consider creatine intake (Allen). For this reason, the distinction between a biomarker change and true renal injury must be made clearly (Antonio et al.; Allen).

Other public fears also need to be separated from evidence. The hair loss concern largely comes from one study involving college-aged rugby players, where CM loading was associated with increased dihydrotestosterone. However, this result has not been consistently repeated in other studies examining creatine and testosterone-related hormones (Antonio et al.). CM is also compared to anabolic steroids in some public discussions because both are associated with

muscle and performance, but this comparison is biologically inaccurate. Anabolic steroids act through hormonal pathways connected to testosterone, whereas CM primarily supports ATP regeneration through the PCr system (Antonio et al.).

Adolescent Evidence and Public Perception

Evidence presented for adolescents is heavily limited when compared to adult-based evidence, making public interpretation more complicated. Available youth data indicate that creatine use exists among middle and high school students, but prevalence varies by age, gender, athletic status, and survey context. In a survey of 1,103 student-athletes aged 10 to 18, 5.6% reported using creatine, with the most common reasons for use being improved athletic performance and appearance. The most common reason for non-use was safety concerns (Metzl et al.). More detailed adolescent-focused reviews report that CM use appears more common among males, older students, and students who weight train (Jagim and Kerksick).

The adolescent safety literature remains extremely cautious, but it does not support treating CM as automatically dangerous for young people. Reviews of adolescent efficacy studies reported limited evidence of any adverse events, and several studies that monitored laboratory markers found no clinically relevant adverse effects (Jagim and Kerksick). CM has also been used medically in pediatric populations with creatine deficiency syndromes, muscular dystrophies, childhood systemic lupus erythematosus, and gyrate atrophy under clinical supervision (Jagim and Kerksick). These examples do not encourage unsupervised supplement use in teenagers. Instead, they demonstrate that adolescent CM use has to be evaluated through medical context and research quality, not through age alone.

Public perceptions become more confusing because caution can be written in broad language for consumers. Some people may interpret under-18 label advisories on CM products as evidence that the supplement is unsafe for adolescents. Nevertheless, Jagim and Kerksick explain that these advisories may be tied to manufacturer liability concerns. Furthermore, they note that hesitation around adolescent use reflects limited youth-specific safety data (Jagim and Kerksick). This is significant to note since students and parents may treat age-based warning labels as proof of danger, even when the warning mainly reflects limited adolescent-specific research and liability concerns.

A major problem is that CM is grouped with performance-enhancing drugs without a scientific basis. Jagim and Kerksick criticize research that poorly categorized CM as an androgenic anabolic agent rather than an amino-acid compound, since that framing of the supplement can create compounding layers of bias (Jagim and Kerksick). This creates “guilt by association” and can make students interpret CM through the language of doping before understanding its actual role in energy metabolism.

Methodology

Ethics Approval

Since this study involved high school students as participants, it followed the York Region Science and Technology Fair (YRSTF) low-risk human participant process. The Participation of Humans in Research Low-Risk Approval Form (LRAF) was completed to classify the project as low risk and record the student researchers, school, and regional STEM fair information.

Before any participant completed the questionnaire, they received two consent documents: the Letter of Information (LOI) and the Permission Form Informed Consent (PFIC). The LOI explained the purpose of the study, the questionnaire format, possible risks and benefits, time commitments, confidentiality, withdrawal rights, and how results would be used. To ensure that all participants felt comfortable and safe, no questionnaire items were forced or mandatory. Participants were informed that they could skip any question that made them feel uncomfortable or stressed. The PFIC was used to collect written consent. Since many participants were minors, the PFIC included spaces for both the participant's printed name and signature and the parent or guardian's printed name and signature. Students were only given access to the questionnaire after the required forms were completed and returned.

All signed LOI and PFIC documents were stored securely with a supervising teacher during the data collection and presentation stages of the project. They were kept separate from the anonymous response data. Electronic questionnaire responses were stored in a password-protected account and were used only for research analysis. Results were reported in aggregate form, and no participant names were visible or included in the data analysis, figures, or final report. After data collection was completed and the forms were no longer required for verification, the signed documents were shredded to protect participant privacy.

Participants and Eligibility

Participants were recruited from a single public high school community in York Region, Ontario, and were considered eligible if they were high school students who had completed the consent process. Since the study focused on general awareness and perceptions of CM within the school community, students could participate even if they were not athletes or creatine users. Students who had never heard of CM were also included because their responses helped measure a realistic sense of awareness regarding the supplement. Students were excluded if they were not high school students or if the required consent documents were incomplete or not returned.

Data Collection

Data were collected using a self-administered Google Forms questionnaire from February 14, 2026, to March 14, 2026. After students turned in the required consent documents, the questionnaire was shared via a QR code. Participants scanned the code and completed the 10-minute questionnaire independently. At the end of the month-long data collection period, 70 responses were collected. One response was excluded because the required consent documentation was incomplete and could not be verified. The final analyzed sample was N = 69.

A voluntary convenience sampling method was used for this study since participants were recruited from an accessible school population and chose whether to participate. This method was practical for this school-based project, but it may have introduced selection bias because the student sample was not randomly selected. Response bias may have also occurred if students with stronger opinions about fitness, supplements, or health were more likely to complete the questionnaire.

Questionnaire Domains

The questionnaire was organized into four research domains, with each domain corresponding to a separate Google Forms section. The table below lists the items included in each domain.

Table 1. Research Domains and Questionnaire Items

Domain	Purpose	Items included
General Information	To describe the participant sample and activity background.	1. What grade are you currently in? 2. What is your age? 3. What is your gender? 4. Do you currently play on a school or community sports team? 5. How many days per week do you engage in physical activity, such as sports or gym?
Awareness and Perceptions	To measure prior awareness, source of exposure, familiarity, and basic understanding of CM.	1. Have you heard of creatine monohydrate before? 2. If yes, where did you first hear about it? 3. On a scale from 1 to 5, how familiar are you with what creatine does? 4. In your own words, what do you think creatine does? 5. Have you heard people compare creatine to anabolic steroids?
Personal Use and Attitudes	To examine previous use, future willingness, factors that influence decision-making, and parent or guardian support for the supplement.	1. Have you ever taken creatine supplements? 2. If not, would you consider trying creatine in the future? 3. What would most influence your decision to take or not take creatine? 4. Do you think your parents or guardians would support creatine use? 5. What information would you need to feel confident in your opinion about creatine?
Stigma and Negative Perception	To collect written explanations about why CM may be viewed negatively.	1. In your opinion, why do you think some people have a negative view of creatine?

Data Analysis

Responses to questionnaire items were analyzed depending on question format. For closed-ended items, frequency counts and percentages were used to summarize the responses. This method was used for variables including grade level, gender, sports participation, prior awareness of CM, source of first exposure, previous CM use, and exposure to steroid comparison. The 1 to 5 familiarity item was interpreted as an ordinal scale, since the responses moved from low familiarity all the way to high familiarity. It was summarized using the average familiarity score calculated by adding all the ratings and dividing it by the total number of responses for that given item.

For open-ended items, responses were manually coded by reviewing each written answer and grouping similar ideas into recurring themes. Coding was used for the questions asking what students believed CM does in the body, what information students would need to feel confident in their opinion about CM, and why some people may have negative views of CM. Certain responses may have reflected more than one idea, so themes were treated as overlapping.

No inferential statistical tests were conducted in this study since a voluntary convenience sample was used. Therefore, the analysis of survey results avoided making claims about all adolescents and only focused on descriptive patterns represented within the collected sample. Survey findings were compared with the literature reviewed to identify where student perceptions aligned with current CM research and where misconceptions were most common.

Results

The final analyzed sample consisted of 69 valid questionnaire responses. Results are presented according to the four research domains described in the methodology.

General Information

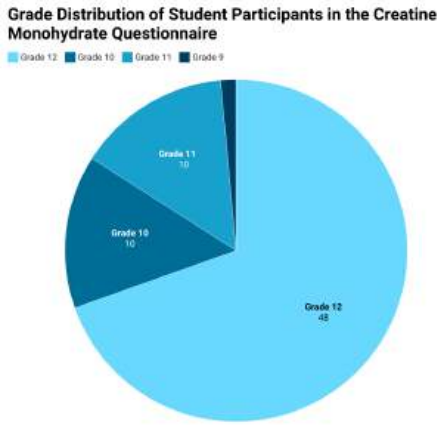


Figure 1A. Grade distribution of participants included in the CM questionnaire. Grade 12 students were the largest subgroup in the sample, accounting for 48 participants (69.6%). Grade 10 and Grade 11 each accounted for 10 participants (14.5% each), and Grade 9 included only 1 participant (1.4%). This illustrates that the sample mainly reflects the perspectives of high school seniors.

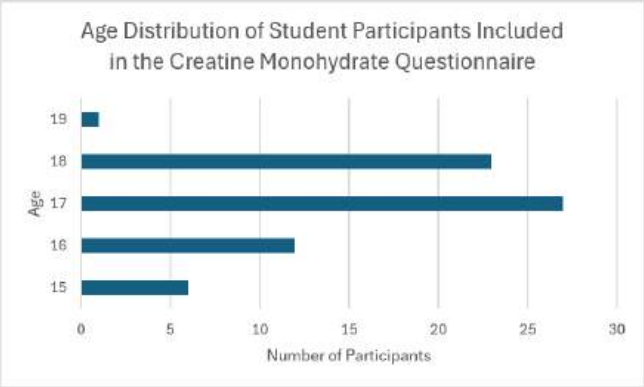


Figure 1B. Age distribution of student participants included in the CM questionnaire. The largest age group was 17-year-olds, represented by 27 participants (39.1%), followed by 18-year-olds with 23 participants (33.3%). Smaller groups included 16-year-olds with 12 participants (17.4%), 15-year-olds with 6 participants (8.7%), and 19-year-olds with 1 participant (1.4%). These results show that most of the voluntary sample was composed of older adolescents.

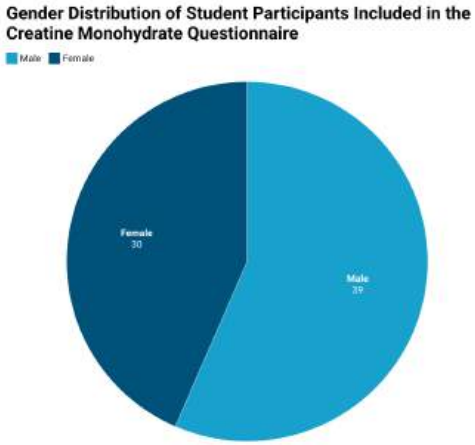


Figure 1C. Gender distribution of participants included in the CM questionnaire. The sample included 39 male participants (56.5%) and 30 female participants (43.5%). Although "Other" was included as a response option, no participants selected it. Overall, the pie chart above shows that both female and male perspectives were represented in the study, with males making up a slightly larger portion of the sample.

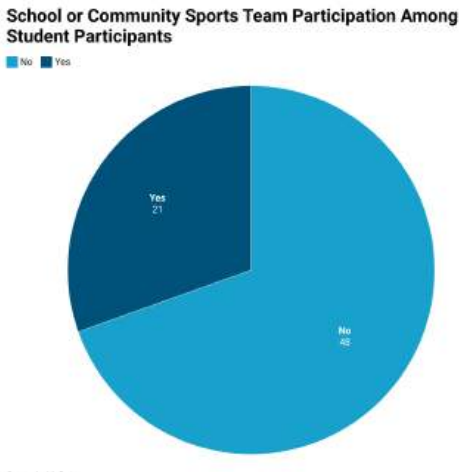


Figure 1D. School or community sports team participation among participants. Most students were not currently involved in a school or community sports team: 48 participants selected No (69.6%), and 21 selected Yes (30.4%). The sample included more students outside organized team sports than students who were currently involved in organized sports.

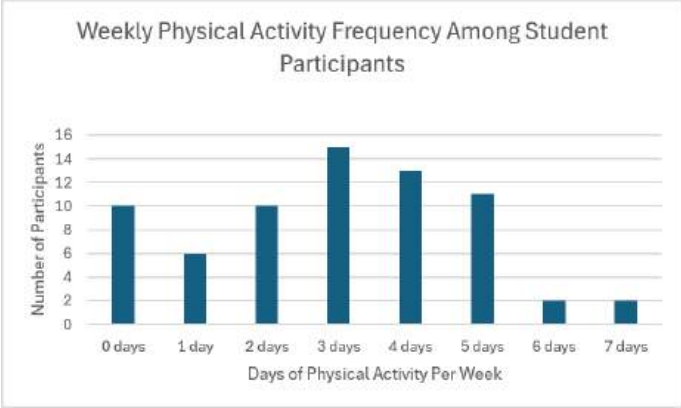


Figure 1E. Weekly physical activity frequency among participants. Physical activity levels among participants ranged from 0 to 7 days per week. The most common response given was 3 days per week, selected by 15 participants (21.7%), followed by 4 days with 13 participants (18.8%) and 5 days with 11 participants (15.9%). Ten participants selected 0 days (14.5%), 10 selected 2 days (14.5%), 6 selected 1 day (8.7%), 2 selected 6 days (2.9%), and 2 selected 7 days (2.9%). These results show that the voluntary sample varied widely in weekly physical activity.

Awareness and Perceptions

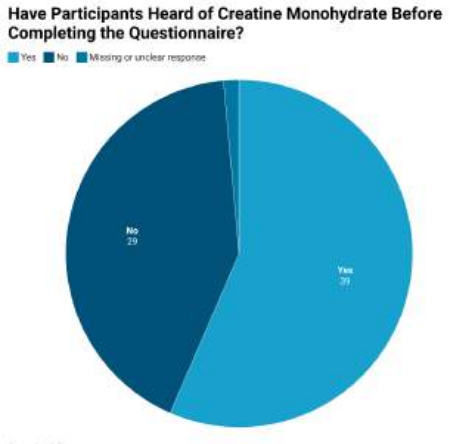


Figure 2A. Prior awareness of CM before completing the questionnaire. Thirty-nine participants (56.5%) reported having previously heard of CM. Another 29 participants (42.0%) noted no prior awareness, and 1 participant (1.4%) did not answer the question.

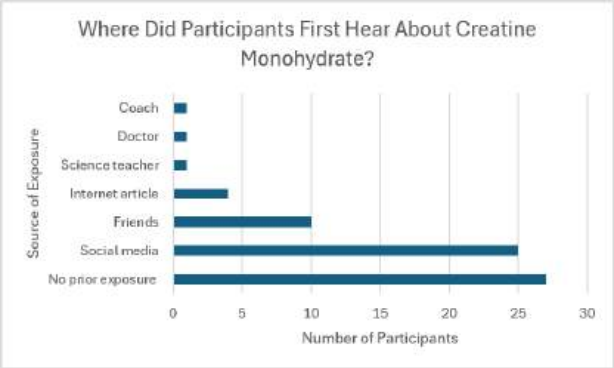


Figure 2B. First reported source of exposure to CM. With 25 participants (36.2%), social media is presented as the most common source of first exposure to CM. Friends were the second most common source, selected by 10 participants (14.5%). Internet articles were selected by 4 participants (5.8%), and a science teacher, doctor, and coach were each reported by 1 participant (1.4%). The remaining 27 participants (39.1%) either had no prior exposure to CM or did not answer this optional question. Overall, students were more likely to encounter CM on informal media sources rather than through academic sources.



Figure 2C. Participant familiarity with the function of CM was measured on a 1 to 5 scale; 1 represented "not at all familiar" and 5 represented "very familiar." Generally, familiarity with CM itself was low on this scale. In fact, the average familiarity rating among usable responses was around 2.3 out of 5, signifying that most participants did not report strong confidence in their understanding of CM. The most common response was a rating of 1, which was selected by 26 participants (37.7%). Ratings of 2, 3, 4, and 5 were selected by 14 participants (20.3%), 13 participants (18.8%), 10 participants (14.5%), and 4 participants (5.8%), respectively. Two participants (2.9%) did not provide a rating.

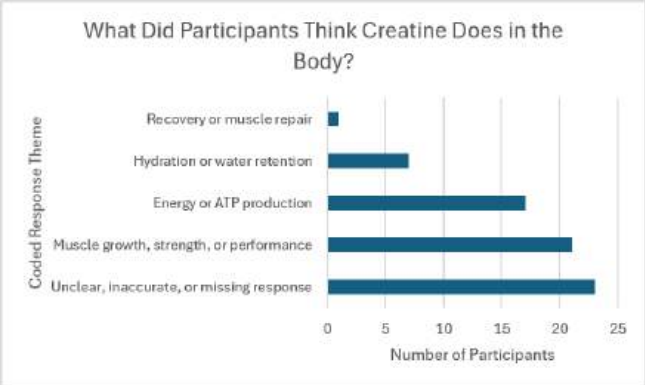


Figure 2D. Coded themes from written responses regarding the physiological function of CM. The open-ended responses in Figure 2D demonstrate that participants had mixed levels of understanding. In total, 23 responses (33.3%) were manually coded as unclear, inaccurate, or missing. However, 21 responses (30.4%) connected CM to muscle growth, strength, or performance, and 17 responses (24.6%) identified ATP production in relation to CM. Smaller groups mentioned hydration or water retention, with 7 responses (10.1%), and recovery or muscle repair, with only 1 response (1.4%). In general, some participants connected CM to exercise and energy, but many of the valid responses still lacked an accurate explanation of the compound.

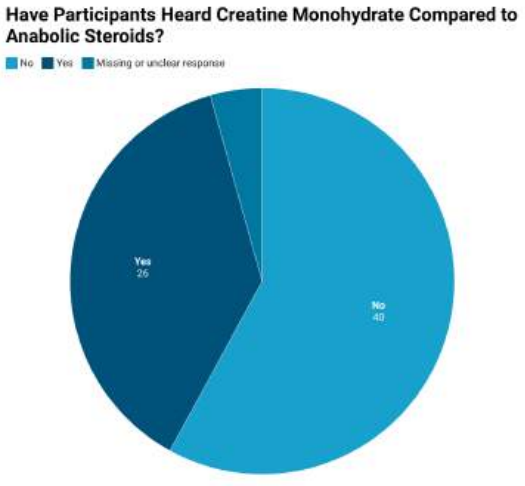


Figure 2E. Student exposure to comparisons between CM and anabolic steroids. Based on the chart above, most participants had not heard CM compared to anabolic steroids. Forty participants (58.0%) selected No, and 26 participants (37.7%) selected Yes. Three participants (4.3%) did not provide a response. Even though the comparison was not reported by the majority of the sample, more than one-third of participants had heard of it, suggesting that steroid-related misconceptions persisted within the sample.

Personal Use and Attitudes

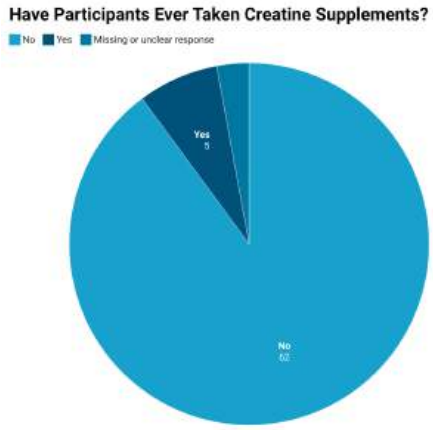


Figure 3A. Previous use of creatine supplements among student participants. Most participants had never taken creatine, with 62 participants (89.9%) selecting No. Five participants (7.2%) reported previous creatine use, and 2 participants (2.9%) did not provide an answer. Through the results from this question, it is evident that the sample mostly represented the perspective of non-CM users.

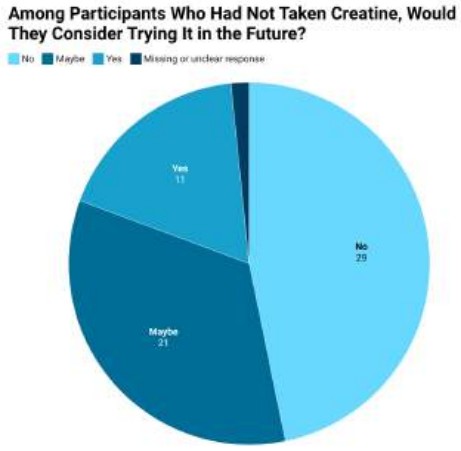


Figure 3B. Future willingness to take CM among participants who had not previously used it. Among the 62 participants who had not taken CM before, 29 participants (46.8%) selected No, meaning they would not consider trying CM in the future. Another 21 participants (33.9%) selected Maybe, and 11 participants (17.7%) selected Yes. One participant (1.6%) from the original 62 did not provide an answer.

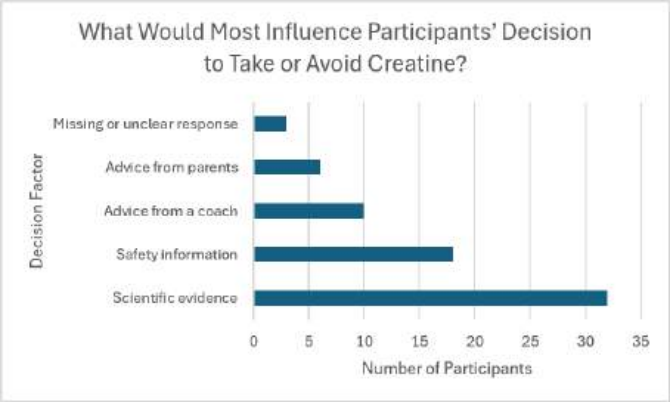


Figure 3C. Factors influencing students' decision regarding CM use. Scientific evidence was the most influential factor in participants' decisions, selected by 32 of the 69 participants (46.4%). Safety information was also important, selected by 18 participants (26.1%). Advice from a coach was selected by 10 participants (14.5%), and advice from parents was selected by 6 participants (8.7%). Three participants (4.3%) had missing or unclear responses. In general, participants placed more emphasis on evidence and safety than on advice from others.

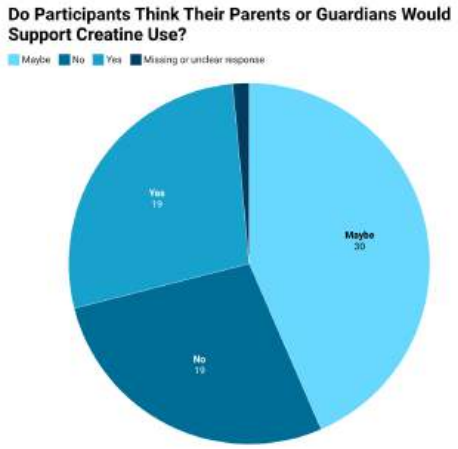


Figure 3D. Perceived parent or guardian support for creatine supplementation. The most common answer was Maybe, which was selected by 30 participants (43.5%). Equal numbers of participants selected No and Yes, with each response selected by 19 participants (27.5%). One participant (1.4%) had a missing or unclear response. These results reveal a divided perception of parent or guardian support for CM intake, with uncertainty being the most common response.

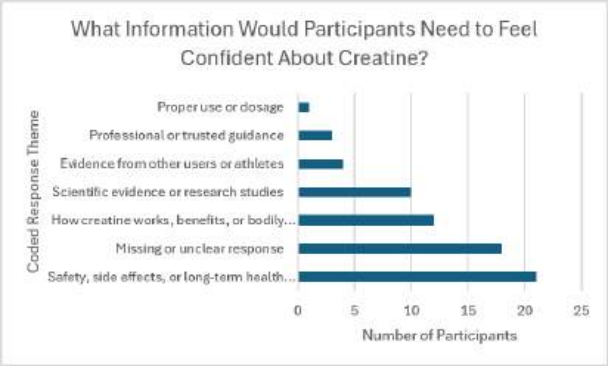


Figure 3E. Information participants needed to feel confident in their opinion about CM. The coded responses show that safety, side effects, or long-term health effects were the most common information needed, appearing in 21 written responses (30.4%). The second largest group was missing or unclear responses, with 18 responses (26.1%). Information regarding how CM works, its benefits, or its effects on the body appeared in 12 responses (17.4%), and scientific evidence or research studies appeared in 10 responses (14.5%). Smaller categories included evidence from other users, with 4 responses (5.8%), professional or trusted guidance, with 3 responses (4.3%), and proper use or dosage, with 1 response (1.4%). As a whole, safety-related information was the most valued by this student sample before forming confident opinions about CM.

Stigma and Negative Perceptions

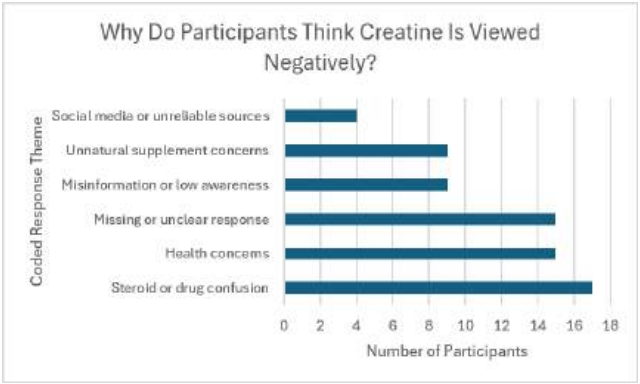


Figure 4A. Written reasons for negative views of CM. Negative perceptions of CM were most commonly linked to confusion with steroids or drugs, which appeared in 17 responses (24.6%). Health concerns appeared in 15 responses (21.7%), and misinformation or low awareness appeared in 9 responses (13.0%). Unnatural supplement concerns also appeared in 9 responses (13.0%), while social media or unreliable sources appeared in only 4 responses (5.8%). Missing or unclear answers made up 15 cases (21.7%), which may have limited how fully the written responses represented student opinions.

Discussion

This study examined high school students' awareness, familiarity, attitudes, and stigma-related perceptions of CM. The central finding is a clear incongruity between CM exposure and CM comprehension. CM circulated within the student population, yet its meaning remained unsettled. The first domain frames this interpretation: Grade 12 students made up

69.6% of participants, and 69.6% of students were not on a school or community sports team. CM awareness was therefore not limited to competitive athletic spaces. It appeared within a broader adolescent fitness culture where students may encounter supplements through casual exercise, peer discussion, and digital media.

The clearest divide appeared between recognition and conceptual fluency. In Figure 2A, prior awareness reached 56.5% among participants, but Figure 2C recorded an average familiarity score of only 2.3 out of 5. Figure 2D gives this gap more substance. Students most commonly connected CM with muscle growth, strength, or performance (30.4%), followed by ATP and energy production (24.6%). These results indicate that many participants understood CM first through its external fitness reputation, then only partially through its biochemical purpose. In other words, CM was familiar as a performance symbol before it was understood as a physiological compound involved in cellular energy metabolism.

Moreover, CM's first exposure pathway was predominantly informal. In Figure 2B, social media accounted for 36.2% of initial exposure, followed by friends at 14.5%. Doctors, coaches, and science teachers each represented only 1.4%, placing expert or educational sources at the margins of student exposure. This distribution situates CM inside a peer- and algorithm-mediated information network, where supplement claims can become compressed, decontextualized, and circulated without scientific arbitration. Students encountered CM through channels capable of making the supplement visible before making it intelligible, which accounts for the divide between prior awareness in Figure 2A and low familiarity in Figure 2C.

Parent and guardian responses reinforced CM's ambiguous status. In Figure 3D, the most common perceived response was Maybe at 43.5%, and Figure 3E recorded safety, side effects, and long-term health impacts as the most requested information at 30.4%. Thus, CM occupied a liminal position between ordinary fitness aid and health-related concern. It was not automatically rejected, but it required justification before trust could fully develop.

The strongest stigma-related mechanism was steroid conflation. Figure 2E recorded that 37.7% of participants had heard CM compared to anabolic steroids, and Figure 4A identified steroid or drug confusion as the leading reason for negative views at 24.6%. Taken together, the findings present CM stigma as the product of partial, socially mediated exposure. The main implication is the need for adolescent supplement literacy that differentiates CM from steroids, clarifies its biological role, and addresses safety concerns without encouraging uncritical supplement use.

Limitations

Several limitations should be made clear when interpreting these findings. First, the sample was relatively small and demographically limited since most participants were in Grade 12. As a result, the findings may reflect senior high school perspectives more strongly than the broader high school population. Second, the study relied heavily on self-reported responses, meaning participants' answers depended on their own recall, confidence, and interpretation of CM-related questions. For example, someone may feel confident in their understanding of CM

and give themselves a 5 as a familiarity score, when in reality they may know little to nothing about its physiological process.

Third, some open-ended questions were left unanswered because participants were not required to complete every item. This method preserved voluntary participation, but it also reduced the amount of written data available for future qualitative analysis in some domains. Future research could make selected open-ended questions mandatory or include follow-up prompts to maximize the number of complete responses. Fourth, the study used descriptive analysis only, meaning the results identified patterns in attitudes, stigma, and awareness but did not establish causal relationships. Finally, this study did not separately analyze differences by gender, sport participation, or physical activity level. A follow-up study could examine whether these factors influence CM familiarity, willingness to use supplements, and susceptibility to steroid-related misconceptions.

Conclusion

In conclusion, this study found that CM occupies an uncertain place in high school students' perceptions. More than half of participants had heard of CM, but familiarity among participants remained low. Student explanations tended to focus on visible outcomes such as muscle growth and physical performance before its biological function. This supports the purpose of the study by identifying a gap between scientific knowledge of CM and the way students understand the supplement in everyday fitness culture.

These findings indicate that student views of CM are influenced less by direct use and more by the information environment surrounding the supplement. Since most participants had never taken CM, their opinions were largely formed through exposure, caution, and association. Social media, peer discussion, safety concerns, and steroid comparisons all contributed to the way CM was interpreted.

By addressing these misconceptions directly, schools and health educators can help students evaluate CM with more scientific precision. Future education should explain CM's role in ATP regeneration, distinguish it from anabolic steroids, and discuss adolescent safety concerns in a balanced way. This study provides an early foundation for understanding how supplement literacy can reduce confusion without promoting careless supplement use.

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Tracking and Forecasting Global Antibiotic Resistance: A Comprehensive Data Analysis and Future Projection Study By Himay Marada

ABSTRACT

Antimicrobial resistance is a top ten global health threat, yet most studies examine single pathogen–antibiotic combinations over short periods of time. This lack of research across the field creates a gap in knowledge. To identify which combinations are at the highest risk, a comprehensive, multi-pathogen analysis is needed. This study analyzes global resistance trends from 2018 to 2023, evaluates the relationship between antibiotic consumption and resistance, and projects high risk pathogen–antibiotic combinations through 2035 under varying intervention scenarios. Resistance data from 2018-2023 for Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, and Mycobacterium tuberculosis were extracted from the WHO GLASS database alongside consumption data from 2016-2023 and were analyzed using R to get descriptive statistics and linear regression data get information about current rates and trends. ARIMA (AutoRegressive Integrated Moving Average) time-series models with 95% confidence intervals were used to forecast resistance rates through 2035 over three scenarios: a baseline scenario, a 15% reduction in annual resistance growth, and a 20% acceleration. Global resistance is projected to increase by 0.67 percentage points per year, reaching an average of 37.2% by 2035. Antibiotic consumption showed a moderate positive correlation with antibiotic resistance ($r = 0.46$), showing that consumption is a contributing factor to resistance. Klebsiella pneumonia's resistance to cefotaxime (59.6%) and ciprofloxacin (51.2%) is projected to exceed the 50% critical threshold. Scenario comparisons revealed a 13-percentage-point difference between aggressive intervention (31.6%) and inaction (44.6%) by 2035. These findings identify high risk pathogen–antibiotic combinations and demonstrate that strengthened antimicrobial stewardship could substantially reduce projected resistance growth.

KEYWORDS

Antimicrobial resistance, antibiotic consumption, ARIMA forecasting, WHO GLASS, Klebsiella pneumoniae

INTRODUCTION

The purpose of this paper is to analyze global antimicrobial resistance (AMR) trends and project future outcomes using a new multi-pathogen approach compared to other single pathogen studies completed previously. Antimicrobial resistance is a top ten global health threat. In 2019 bacterial antimicrobial resistance was directly responsible for 1.27 million deaths globally, which was more than HIV and malaria combined (Antimicrobial Resistance Collaborators, 2022). Economic projections show that without intervention AMR could cost the global economy $100 trillion by 2050, and annual deaths could reach 10 million, surpassing cancer as a leading cause of mortality (O'Neill, 2016). When resistance rates exceed 50% when testing empirically,

treatments become ineffective. This forces physicians to delay effective treatment or resort to broader-spectrum, more toxic alternatives also known as second line antibiotics. For critically ill patients with sepsis, each hour of delayed appropriate antibiotic therapy increases mortality risk by 7.6% (Kumar et al., 2006). Identifying which pathogen-antibiotic combinations will cross this critical threshold therefore shows providers and corporations where to target funds and resources to prevent antibiotic crises. Despite AMR's urgency, existing research exhibits three critical limitations. First, most studies examine individual pathogen-antibiotic pairs in isolation rather than conducting multi-pathogen analyses. Second, forecasting windows are usually around 2-3 years and. This is not a large enough window of data for policy makers to act on. Third, tuberculosis (TB) resistance patterns differ fundamentally from bacterial resistance patterns due to TB's need for multiple treatments compared to other bacterial diseases. This prevents TB from being easily integrated into policies and studies regarding antibiotic resistance due to this fundamental difference. This research addresses these gaps through four specific research questions: (1) How have global AMR rates changed from 2018 to 2023 (2) What is the relationship between antibiotic consumption and resistance development? (3) Which pathogen-antibiotic combinations will exceed 50% resistance by 2035 under current trends? (4) How much can intervention strategies alter these trajectories? By answering these questions, this research provides policymakers with evidence-based targets to be able to accurately figure out where to allocate funds and resources This study hypothesizes that if current consumption trends continue without intervention, at least one pathogen-antibiotic combinations will exceed the 50% critical resistance threshold by 2035, and that by reducing antibiotic consumption, resistance will also be reduced or mitigated

METHODOLOGY

Data Sources and Collection All data used in this study were obtained from the World Health Organization's Global Antimicrobial Resistance Surveillance System (GLASS), which provides standardized resistance and antimicrobial use surveillance data from participating countries (World Health Organization, 2023). Resistance observations were organized by pathogen, antibiotic or resistance category, country, and year before analysis. GLASS uses standardized laboratory approaches based on Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) methods, allowing observations from participating countries to be compared using a common framework. The analysis used the resistance percentages and consumption measures available in the selected GLASS records rather than attempting to impute unreported resistance values. Organization's Global Antimicrobial Resistance Surveillance System (GLASS), which combines standardized resistance and consumption data from participating countries(World Health Organization, 2023). GLASS employs consistent laboratory methodologies across countries using Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) measures to ensure data can be compared across regions. The analysis covered four pathogen groups or resistance dimensions. Bacterial resistance data included

Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus across 15 antibiotic classes from 48 countries. Tuberculosis data were represented separately through multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) observations from 89 countries because TB resistance is measured and treated differently from the other bacterial pathogens. Antibiotic-consumption data were measured as defined daily doses per 1,000 inhabitants per day (DDD/1,000/day) from 48 countries. The resistance dataset covered 2018–2023, while consumption data extended from 2016–2023. Records were grouped by pathogen-antibiotic combination and year for analysis, and observations without the variables required for a particular calculation were excluded from that calculation rather than assigned estimated values. The final dataset contained 2,470 records: 1,380 bacterial resistance records, 1,090 tuberculosis resistance records, and 403 consumption records. Statistical Analyses Descriptive statistics, including mean, median, standard deviation, and range, were calculated for each pathogen-antibiotic combination. The mean was used to summarize the average resistance percentage, while the median provided a measure less affected by unusually high or low observations. Standard deviation and range were used to quantify how widely resistance varied among observations. These calculations were performed before forecasting so that combinations with consistently high resistance and combinations with substantial country-to-country variation could be identified. Pearson correlation coefficients were calculated to evaluate the direction and strength of the linear relationship between antibiotic consumption and resistance percentage for each selected pathogen-antibiotic combination. The correlation coefficient (r) ranges from -1 to +1, with positive values indicating that higher consumption was associated with higher resistance. Statistical significance was assessed using two-tailed tests with alpha = 0.05. Because correlation measures association rather than causation, the results were interpreted as evidence of a relationship between consumption and resistance rather than proof that consumption alone caused changes in resistance. Where variance explained by the linear association was discussed, it was represented by r^2 rather than r. Multiple linear regression was then used to estimate the relationship between resistance, antibiotic consumption, and year using the model Resistance% = B0 + B1(Consumption) + B2(Year) + e. B0 represents the estimated baseline resistance, B1 represents the estimated percentage-point change in resistance associated with a one-unit increase in consumption, B2 represents the estimated annual change associated with year after accounting for consumption, and e represents unexplained variation. Including both consumption and year allowed the analysis to distinguish the association with consumption from an underlying time trend. Model fit was evaluated using R^2, while coefficient estimates were interpreted as associations rather than causal effects. Regression assumptions and influential observations were checked using residual plots, variance inflation factors (VIF < 5), and Cook's distance. Residual plots were used to look for major departures from constant variance or systematic patterns in the residuals. VIF was used to check for problematic multicollinearity between the predictor variables, with values below 5 treated as acceptable for this analysis. Cook's distance was used to identify observations that could have an unusually large influence on the fitted regression model. These checks were performed before interpreting the regression

coefficients and statistical relationships. ARIMA (AutoRegressive Integrated Moving Average) models were used to project resistance rates from 2024 through 2035 for each pathogen-antibiotic combination. For each combination, observations were ordered chronologically by year and the available historical resistance series was used as the forecasting input. The auto.arima() function in R selected the model specification using Akaike Information Criterion (AIC), balancing goodness of fit against model complexity. Forecasts were generated annually through 2035 and reported with 95% confidence intervals. Because resistance is expressed as a percentage, projected values were constrained to the feasible 0–100% range. The 50% threshold was used as a descriptive benchmark to identify combinations whose baseline forecasts were projected to exceed 50% by 2035; crossing this threshold was not treated as a statistical test of treatment failure. Forecast performance was evaluated using Ljung-Box tests, mean absolute percentage error (MAPE), and out-of-sample testing. The Ljung-Box test assessed whether meaningful autocorrelation remained in model residuals. MAPE quantified the average percentage difference between predicted and observed values, with lower values indicating better predictive accuracy. For out-of-sample validation, models were fitted using 2018–2021 observations and then used to forecast 2022–2023; these forecasts were compared with the observed 2022–2023 values. This provided a check on performance using observations not used during model fitting. Forecast uncertainty was reported using 95% confidence intervals, and all analyses were conducted in R version 4.3.0. Finally, three intervention scenarios were used to examine how different consumption trajectories could alter projected resistance. The baseline scenario continued the historical trend without an additional intervention adjustment. The best-case scenario modeled a 15% reduction in antibiotic consumption, representing the potential effect of stronger antimicrobial stewardship programs. The worst-case scenario modeled a 20% increase in consumption, representing continued or worsening consumption trends. The resulting scenarios were compared using the projected 2035 resistance rates, allowing the analysis to quantify the difference between continued current trends and alternative intervention conditions. These scenarios were intended as projections of possible trajectories rather than guarantees of the effect of a specific policy. ARIMA (AutoRegressive Integrated Moving Average) models were used to project resistance rates from 2024-2035 for each pathogen-antibiotic combination. Model selection employed the auto. arima() function in R,which selected optimal specifications based on Akaike Information Criterion (AIC). Forecasts included 95% confidence intervals, with upper bounds for projections capped at 100% to ensure all values were possible, removing skews in trends due to impossible outcomes. The model was validated by employing Ljung-Box tests, mean absolute percentage error (MAPE), and out-of- sample testing using 2018-2021 data to forecast 2022-2023. Three scenarios modeled policy impact and intervention scenarios:A baseline continuing current trends; best-case scenario reducing consumption by 15% through enhanced stewardship programs; and worst-case scenario increasing consumption by 20%, reflecting trends in some low and middle income countries. All analyses were conducted in R version 4.3.0.

RESULTS

Global Resistance Trends 2018-2023 Across all four pathogens mean resistance increased from 33.1% in 2018 to 36.5% in 2023 representing a 3.4 percentage point absolute increase or 10.3% relative increase over five years . This translates to an average annual growth rate of 0.67 percentage points per year which is significant. However, pathogen-specific patterns varied substantially, E. coli resistance increased from 28.2% to 31.6% (+3.4 pp), K. pneumoniae from 41.8% to 47.3% (+5.5 pp) and S. aureus (MRSA) declined slightly from 31.2% to 28.9% (-2.3 pp) likely reflecting successful MRSA control programs in high-income countries. Tuberculosis resistance patterns differed. MDR-TB rates in new cases increased from 7.8% to 8.3% (+0.5 pp) while XDR-TB increased from 3.2% to 3.6% (+0.4 pp). Although these percentages appear lower than bacterial resistance each case represents profound clinical and public health challenges, MDR-TB requires 18-24 months of treatment with second-line drugs causing severe side effects costs $50,000-200,000 per patient and achieves cure rates of only 55-60%. XDR-TB is even more dire with cure rates below 50% and extremely limited treatment options. However, due to tuberculosis's differing treatment methods, public health programs and social factors these results are more inaccurate with an r value of 0.28. Standard deviation analysis revealed large regional differences. For K. pneumoniae and third-generation cephalosporins resistance ranged from 18.2% (Sweden) to 71.3% (Egypt) with SD = 22.4% indicating massive country-to-country variation. This differences suggests that uniform global interventions would be inefficient and ineffective. The biggest solution would be customized regional strategies accounting for local healthcare infrastructure prescribing practices and infection control capacity. Relationship Pearson correlation analysis performed across eight pathogen-antibiotic combinations showed statistically significant positive relationships in seven cases The overall mean correlation was $r = 0.46$ ($p < 0.001$) confirming that countries with higher antibiotic consumption rates are likely to have higher resistance rates. The strongest correlation was E. coli with carbapenems ($r = 0.69$, $p < 0.001$) indicating that 69% of the variation in resistance moves in tandem with consumption variation, this is a strong relationship. Linear regression models quantified these relation- ships precisely. For E. coli and carbapenems the equation Resistance% = 12.5 + 1.8(Consumption) + 0.4(Year) demonstrated that each 1 DDD/1000/ day increase in consumption predicts 1.8 percentage point higher resistance, time alone contributes 0.4 percentage points annually likely reflecting factors beyond consumption such as international travel and climate change and $R^2 = 0.47$ indicates consumption and time together explain 47% of resistance variation with the remaining 53% attributable to unmeasured factors including hospital infection control healthcare quality and patient demographics. These findings support the potential importance of antimicrobial stewardship as an intervention targeting antibiotic consumption. However, because the analysis is observational and includes a limited set of predictors, the consumption-resistance relationship should be interpreted as an association rather than direct evidence of causation. as an evidence-based intervention. If a country reduces consumption from 20 to 15 DDD/1000/day (a 25% reduction) resistance would decrease by $1.8 \times 5 = 9$ percentage points. ARIMA forecasting identified two pathogen-antibiotic combinations projected to exceed the 50% critical threshold

by 2035 under baseline conditions. First K. pneumoniae with third-generation cephalosporins (primarily cefotaxime and ceftriaxone) is projected to reach 59.6% resistance (95% CI: 54.1-65.1%) by 2035. This represents a 12.3 percentage point increase from 47.3% in 2023 an annual growth rate of 1.03 percentage points. The Ljung-Box test for this model yielded $Q = 7.2$, $p = 0.41$ confirming no residual autocorrelation and validating model adequacy. Second K. pneumoniae with fluoroquinolones (ciprofloxacin levofloxacin) is projected to reach 51.2% resistance (95% CI: 46.8-55.6%) by 2035 increasing from 43.7% in 2023 at 0.63 percentage points annually. Again Ljung-Box validation ($Q = 6.8$, $p = 0.34$) confirmed model reliability so we can trust these projections. The convergence of both critical combinations on K. pneumoniae is highly significant. This Gram-negative bacterium causes serious health- care-associated infections including ventilator-as- sociated pneumonia catheter-related bloodstream infections and intra-abdominal sepsis, primarily affecting immunocompromised patients undergoing chemotherapy organ transplantation or intensive care. When first-line cephalosporins fail clinicians escalate to carbapenems which are our last-resort broad-spectrum antibiotics. If carbapenem resistance subsequently develops (currently 14.2% for K. pneumoniae but rising at 0.8 pp/year) extremely limited options remain: colistin (nephrotoxic) tigecycline (limited efficacy) and experimental agents with uncertain effectiveness. Notably, no E. coli or S. aureus combinations exceeded 50% in baseline projections with maximum values of 45.1% (E. coli-cephalosporins) and 30.5% (MRSA) respectively. For tuberculosis MDR-TB is projected at 9.2% and XDR-TB at 4.8% by 2035, well below 50% but still representing thousands of patients facing nearly untreatable infections requiring prolonged toxic therapy .Model Validation and Accuracy Mean absolute percentage error (MAPE) across all ARIMA models was 8.3% indicating that forecasts changed from actual historical values by an average of 8.3%. This shows excellent accuracy for a model (MAPE < 10%) established by forecasting literature (Hyndman & Koehler, 2006). Out-of- sample validation using 2018-2021 data to predict 2022-2023 yielded MAPE = 9.1% confirming that models generalize well to unseen data rather than simply overfitting historical patterns, which is important for making sure the predictions are reliable. Ljung-Box tests for all models produced p-values ranging from 0.28 to 0.52 all substantially above the $\alpha = 0.05$ threshold. This confirms that residuals exhibit no autocorrelation, no patterns remain unexplained by the models and are caused due to chance, validating that ARIMA model Scenario Analysis: Policy Impact Quantification Scenario modeling demonstrated substantial malleability of 2035 outcomes based on intervention choices made over the next 12 years. Under the best-case scenario, comprehensive global antimicrobial stewardship programs reducing con- sumption by 15%, average 2035 resistance would be 31.6% compared to 37.2% baseline and 44.6% worst-case. This 13-percentage point range between best and worst cases proves that policy choices over the next decade will fundamentally determine AMR trajectories. Most critically the best-case scenario prevents K. pneumoniae-cephalosporin resistance from crossing 50% with projections of 50.7% (95% CI: 46.1- 55.3%), just barely maintaining effectiveness. This finding shows 2024-2035 as a critical intervention period. Taking aggressive action will be the only way to mitigate a future antibiotic crisis. The modeled resistance differences

demonstrate potentially important differences between policy scenarios, but the present analysis does not directly estimate mortality or healthcare costs. Translating resistance projections into deaths or economic costs would require additional epidemiological and economic models incorporating infection incidence, attributable mortality, treatment effectiveness, healthcare utilization, and other factors. Therefore, the scenario analysis is interpreted here in terms of projected resistance rates rather than direct estimates of deaths or financial savings. Baseline projections suggest 2.8 million AMR attributable deaths annually by 2035 based on proportional scaling from current 1.27 million deaths and resistance growth rates. The best-case scenario would prevent approximately 180,000 deaths annually, equivalent to eliminating malaria deaths, while the worst-case would add 240,000 deaths beyond baseline

DISCUSSION

The novelty of this project lies in its comprehensive, multi-pathogen approach and the integration of ARIMA time-series forecasting with consumption-resistance correlation analysis. The convergence of both critical combinations on K. pneumoniae clarifies intervention priorities. Evidence-based strategies include enhanced infection prevention and control in healthcare settings, particularly intensive care units where K. pneumoniae transmission occurs primarily through healthcare worker hands and contaminated equipment. Studies demonstrate that multimodal interventions can reduce K. pneumoniae healthcare associated infections by 40-60% (Tacconelli et al., 2018). Rapid diagnostic stewardship and antimicrobial stewardship programs restricting inappropriate cephalosporin and fluoroquinolone use are also crucial, with audit and feedback interventions having reduced broad spectrum antibiotic use by 20-30% without adversely affecting patient outcomes (Davey et al., 2017). Although MDR-TB and XDR-TB percentages remain below bacterial resistance thresholds, tuberculosis resistance requires fundamentally different interventions due to TB's unique transmission dynamics and treatment requirements. Effective TB resistance control requires active case finding through contact tracing, directly observed therapy (DOT) ensuring treatment adherence, and rapid molecular diagnostics. The distinction between bacterial AMR requiring consumption reduction and infection control versus TB requiring case finding and treatment adherence underscores why integrated multi-pathogen analysis aids resource allocation, as both threats require investment but fundamentally different infrastructure. Several limitations should be considered when interpreting these findings. First, GLASS participation is voluntary, and the subset of countries with sufficiently complete data may not represent the global population equally. Underrepresentation of low and middle income countries may introduce geographic and surveillance related bias. Second, the resistance series spans only 2018–2023, providing a relatively short historical window for a forecast extending through 2035; uncertainty therefore increases as the forecast horizon becomes longer. Third, the regression analysis includes a limited number of predictors and cannot account for all factors that influence resistance, including prescribing practices, infection control capacity, healthcare access, population characteristics, and pathogen transmission dynamics. Fourth, Pearson correlation and linear

regression identify associations and do not establish causality. Fifth, ARIMA forecasts cannot anticipate unexpected interventions, emerging resistance mechanisms, major changes in antibiotic use, pandemics, or other structural changes. Finally, the intervention scenarios are counterfactual projections based on specified consumption changes rather than experimentally measured effects of real world stewardship programs. These limitations mean that the forecasts should be interpreted as planning scenarios rather than precise predictions of future resistance. the smaller number of predictors used and the fact that GLASS participation is voluntary, with only 48 countries providing complete data. Low and middle income countries are underrepresented, potentially biasing results. Additionally, ARIMA models assume future trends follow historical patterns and cannot anticipate transformative interventions or catastrophic accelerants. In the future, analyses should expand to 10 or more pathogens as GLASS coverage improves,incorporate machine learning models capturing nonlinear relationships, and integrate genomic resistance mechanism data to understand why some regions show faster resistance spread than others.

FUTURE RESEARCH

Future research should expand the scope of this analysis as additional standardized AMR surveillance data become available. Increasing the number of pathogens and countries represented would improve the global coverage of the analysis, particularly for low and middle income countries that are currently underrepresented in the GLASS dataset. Longer time series would also provide additional information for forecasting models and could help determine whether the trends identified in this study persist over time. Future studies could also compare ARIMA with machine learning and other nonlinear forecasting approaches to determine whether they capture changes in resistance that traditional time-series models may miss. Integrating genomic resistance mechanism data could further explain why resistance spreads more rapidly in some regions or pathogen-antibiotic combinations than others. Additional predictors, such as healthcare capacity, prescribing practices, infection control measures, and regional antibiotic-use patterns, could also be incorporated to improve explanatory power. Finally, future research could evaluate intervention scenarios using more detailed regional models rather than applying global percentage changes, allowing policymakers to assess which strategies are most appropriate for specific healthcare settings.

CONCLUSION

Overall, the integrated analysis identified two K. pneumoniae pathogen-antibiotic combinations projected to cross the 50% resistance benchmark by 2035 under baseline trends, with third-generation cephalosporins reaching 59.6% and fluoroquinolones reaching 51.2%. Scenario analysis showed materially different projected resistance levels under alternative antibiotic-consumption trajectories. The consumption-resistance analysis also identified a positive association between antibiotic use and resistance across the combinations examined. However, the results should be interpreted in light of the study’s limited predictors, geographic

coverage, short historical resistance series, and uncertainty inherent in long range forecasting. The study therefore provides a framework for identifying high risk combinations and evaluating possible future trajectories rather than a direct prediction of future mortality or economic burden. The trends identified establish that two K. pneumoniae combinations will cross the critical 50% resistance threshold by 2035 under baseline trends, with third-generation cephalosporins reaching 59.6% and fluoroquinolones reaching 51.2%. However, scenario analysis demonstrates that enhanced stewardship programs implemented over the next 12 years could reduce 2035 resistance by 5.6 percentage points, potentially preventing 180,000 deaths annually and avoiding $800 billion in healthcare costs. The drop in accuracy between laboratory confirmed and projected values may be attributed to the slight variation that predicted predictor values have from their true values. The findings support continued attention to antimicrobial stewardship, infection prevention, surveillance, and pathogen-specific resistance control. The convergence of projected high resistance in K. pneumoniae highlights a potential priority for surveillance and intervention, while the distinct treatment and transmission characteristics of tuberculosis support maintaining TBspecific strategies. Regional variation also indicates that future interventions may need to account for differences in healthcare infrastructure, antibiotic use, diagnostic capacity, and surveillance coverage. Expanding GLASS participation and improving data completeness would strengthen future global analyses. Antimicrobial stewardship works, as proven by the r = 0.46 consumption-resistance correlation and European success stories. Regional variation, with the Americas growing 44 times faster than Europe, demands customized strategies accounting for local healthcare infrastructure, economic resources, and cultural contexts rather than uniform global mandates. Three policy priorities emerge: immediate focus on K. pneumoniae through infection prevention, rapid diagnostics, antimicrobial stewardship, and vaccine development; region specific intensive support for high growth areas including surveillance infrastructure and diagnostic capacity; and enhanced GLASS participation to improve global coverage from 48 to 150+ countries. The 2024-2035 window is critical. Aggressive action now can preserve antibiotic classes, but delay may render them irreversibly ineffective.

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Targeted Nanoparticle Drug Delivery for Acute Myeloid Leukemia By Sejal Thatipamula

Abstract

Acute Myeloid Leukemia (AML) is an aggressive cancer of the blood and bone marrow that rapidly disrupts normal blood cell production. Traditional chemotherapy remains the standard treatment for AML but often causes severe side effects because it damages both cancerous and healthy cells. Additionally, leukemia cells may survive within the protective bone marrow microenvironment, increasing the risk of treatment resistance and disease relapse. Targeted nanoparticle drug delivery offers a promising strategy to improve treatment specificity and reduce toxicity.

This project proposes a CD33-targeted poly(lactic-co-glycolic acid) (PLGA) nanoparticle carrying Cytarabine as a therapeutic payload for AML treatment. CD33 is highly expressed on approximately 85–90% of AML cells while showing limited expression on normal hematopoietic stem cells, making it an ideal target for selective drug delivery. Anti-CD33 monoclonal antibodies attached to the nanoparticle surface function as targeting ligands that recognize CD33 receptors through a lock-and-key mechanism. Upon binding, the AML cell internalizes the nanoparticle through receptor-mediated endocytosis, allowing controlled intracellular release of Cytarabine.

The proposed nanoparticle design combines the biocompatibility and biodegradability of PLGA with the clinical relevance of Cytarabine and the specificity of anti-CD33 antibodies. This targeted approach has the potential to increase drug concentration within leukemia cells while minimizing damage to healthy tissues. Although several limitations remain, including immune system clearance, bone marrow penetration challenges, manufacturing complexity, and off-target effects, the design is scientifically feasible and supported by existing biomedical research. Continued advancements in nanomedicine may further improve the safety and effectiveness of targeted therapies for AML patients.

Introduction

Acute Myeloid Leukemia (AML) is an aggressive cancer of the blood and bone marrow. It begins when immature myeloid cells, called blasts, develop genetic mutations that cause them to grow and divide at an uncontrollable rate (NCI 2019). These abnormal cells crowd out healthy blood-forming cells, reducing the body's ability to produce normal red blood cells, white blood cells, and platelets. As a result, patients often experience fatigue, frequent infections, easy bruising, and bleeding. AML progresses rapidly and requires immediate treatment, making it one of the most challenging blood cancers to manage.

Healthy cells grow, divide, and die in a controlled manner to maintain normal tissue function. In contrast, cancer cells ignore normal growth signals, avoid programmed cell death (apoptosis), and continue multiplying. Unlike many cancers that form solid tumors, AML develops primarily in the bone marrow. The bone marrow microenvironment acts as the leukemia microenvironment, where blood vessels, stromal cells, immune cells, and signaling

molecules interact with leukemia cells. This environment protects leukemia cells from chemotherapy and contributes to drug resistance. While solid tumors often have acidic pH and abnormal blood vessel formation that influence drug delivery, AML presents a different challenge because leukemia cells circulate throughout the bloodstream and hide within the protective bone marrow niche.

Traditional chemotherapy remains the standard treatment for AML. Although it can destroy rapidly dividing leukemia cells, it also damages healthy bone marrow cells that are essential for producing normal blood cells. This often causes severe side effects, including infections, anemia, bleeding complications, fatigue, and weakened immunity. Another major challenge is that some leukemia cells survive treatment within the bone marrow, increasing the risk of relapse. Because chemotherapy circulates throughout the entire body, it cannot selectively target only cancer cells, resulting in significant toxicity to healthy tissues.

Researchers are studying several nanoparticle systems to improve AML treatment. Liposomes are tiny lipid-based vesicles that protect chemotherapy drugs and improve their delivery while reducing damage to healthy cells. Polymeric nanoparticles are biodegradable carriers that can provide controlled drug release and be engineered to recognize specific leukemia cell markers. Dendrimers are highly branched nanoparticles with multiple surface attachment sites that allow drugs and targeting molecules to be delivered simultaneously. Scientists are also investigating nanoparticles that target proteins commonly found on AML cells, such as CD33 (a protein) and CD123 (Cell Surface protein), to improve precision of the treatment (Johnson et. al 2022).

Targeted nanoparticle drug delivery offers a promising alternative to conventional chemotherapy because nanoparticles can be designed to recognize leukemia cells while avoiding many healthy cells. This targeted approach may increase drug concentration where it is needed most, reduce harmful side effects, and improve treatment effectiveness. Nanoparticles may also help deliver drugs into the bone marrow microenvironment, where chemotherapy-resistant leukemia cells often remain after treatment. Continued research in nanomedicine has the potential to improve survival rates and quality of life for patients with AML by making cancer therapy more precise and less toxic.

Design Specifications

Nanoparticle Shell Material

Why PLGA?

PLGA is one of the most widely used biodegradable polymers in drug delivery because:

- It's biocompatible and biodegradable.
- It protects drugs from degrading in the bloodstream.
- It allows controlled drug release.
- It is FDA-approved for many medical applications.
- Researchers commonly use it in nanoparticle drug delivery systems.

Therapeutic Payload

Why Cytarabine?

Cytarabine is one of the standard chemotherapy drugs used to treat AML. Since it is already used clinically for AML patients, it makes sense to incorporate it into your nanoparticle design.

Targeting Ligand

Target Identification

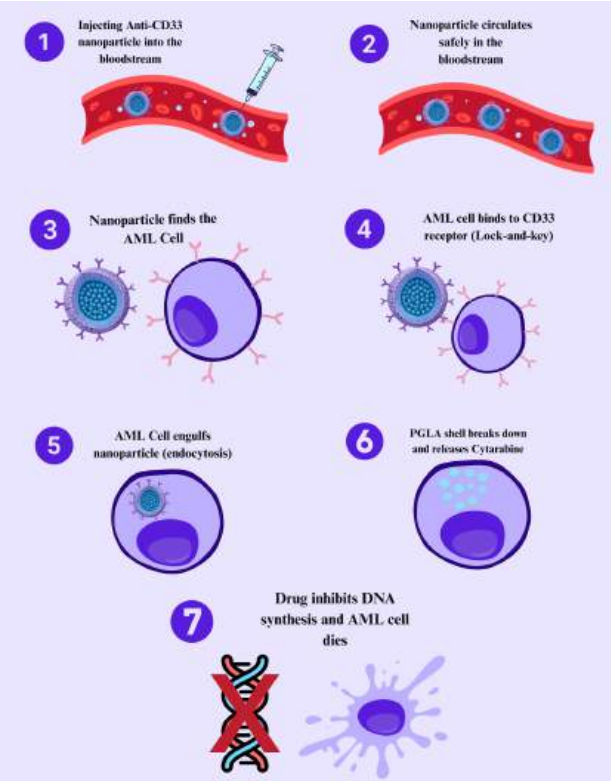
Acute Myeloid Leukemia (AML) cells commonly overexpress a surface receptor known as CD33, making it an excellent target for nanoparticle drug delivery. CD33 is a transmembrane glycoprotein that belongs to the sialic acid-binding immunoglobulin-like lectin (Siglec) family. Approximately 85–90% of AML patients express CD33 on the surface of their leukemia cells, while normal hematopoietic stem cells express little or no CD33. Because of this difference, CD33 allows researchers to distinguish leukemia cells from many healthy blood-forming cells (Wu et. al 2018).

The high expression of CD33 does not directly cause AML, but leukemia cells produce significantly more of this receptor than healthy cells. This overexpression provides an opportunity for targeted therapy because nanoparticles can be engineered to recognize CD33 and deliver chemotherapy directly to leukemia cells while reducing exposure to healthy tissues.

Ligand Identification

The ligand selected for this project is an anti-CD33 monoclonal antibody.

Monoclonal antibodies are laboratory-produced proteins specifically designed to recognize one target molecule. The anti-CD33 antibody binds only to the CD33 receptor found on AML cells with very high specificity. Because antibodies naturally recognize unique molecular structures, they function as highly selective targeting molecules for nanoparticle drug delivery.



Researchers attach the anti-CD33 antibody to the surface of nanoparticles containing chemotherapy drugs. The antibody guides the nanoparticle through the bloodstream until it encounters AML cells expressing CD33.

Lock-and-Key Mechanism

In this model:

- The CD33 receptor acts as the lock. (Step 3)
- The anti-CD33 antibody acts as the key. (Step 3)
- Only the correctly shaped antibody can fit the receptor. (Step 4)

When the nanoparticle reaches an AML cell, the antibody recognizes and binds tightly to the CD33 receptor on the cell surface. This binding signals the leukemia cell to engulf the nanoparticle through a process called receptor-mediated endocytosis. Once inside the cell, the nanoparticle breaks down and releases the chemotherapy drug directly into the leukemia cell.

This targeted delivery increases the concentration of medicine inside cancer cells while reducing damage to healthy cells.

Why CD33?

CD33 was selected because it is highly expressed on most AML cells but has limited expression on normal blood-forming stem cells. This allows nanoparticles to selectively target leukemia cells, improving treatment precision. Targeting CD33 may reduce the harmful side effects associated with conventional chemotherapy by limiting drug exposure to healthy tissues. Additionally, CD33 has already been validated as an important therapeutic target through existing AML treatments, making it one of the most promising receptors for nanoparticle-based drug delivery.

Methods

Step 1 (Injection): The nanoparticle is injected into the bloodstream.

Step 2 (Circulation): The PLGA nanoparticle circulates throughout the body while protecting Cytarabine from premature degradation.

Step 3 Target Recognition): The Anti-CD33 antibodies attached to the nanoparticle recognize CD33 receptors on AML cells.

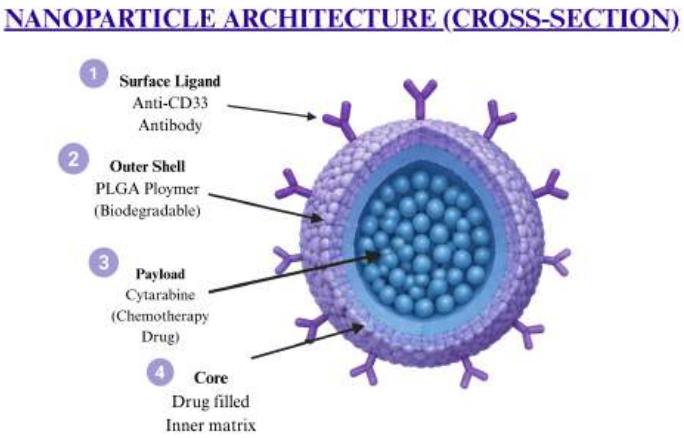
Step 4 (Lock-and-Key Mechanism): The antibodies bind specifically to CD33 receptors found on AML cells.

Step 5 (Cellular Uptake): The AML cell pulls the nanoparticle inside through receptor-mediated endocytosis.

Step 6 (Drug Release): Once inside the leukemia cell, the PLGA shell begins to biodegrade and releases Cytarabine directly into the cancer cell.

Step 7 (Cancer Cell Death): Cytarabine interferes with DNA synthesis in rapidly dividing leukemia cells, causing them to die while minimizing damage to healthy cells.

Nanoparticle Schematic



(Schematic representation of the CD33-targeted PLGA nanoparticle carrying Cytarabine for targeted drug delivery in Acute Myeloid Leukemia cells.)

Limitations and Future Directions

Although the CD33-targeted PLGA nanoparticle carrying Cytarabine shows promise as a targeted treatment for Acute Myeloid Leukemia (AML), there are several limitations that must be considered before it could become a viable clinical therapy.

1. Immune System Clearance
   One major challenge is that the body's immune system may recognize the nanoparticle as a foreign object and remove it from the bloodstream before it reaches AML cells. If the

nanoparticle is destroyed too early, the drug will not be delivered effectively, reducing the treatment's success. Scientists are currently researching surface modifications that help nanoparticles evade immune detection and remain in circulation longer. (Kebede et. al 2021).

2. Limited Specificity of CD33
   Although CD33 is highly expressed on AML cells, it is also present on some healthy myeloid cells. This means that the anti-CD33 antibody may occasionally bind to healthy cells, causing unintended side effects such as temporary damage to normal blood cells and weakened immune function. While targeted therapy is more selective than traditional chemotherapy, it is not perfectly specific.

3. Bone Marrow Penetration
   AML cells often reside within the bone marrow microenvironment, which protects them from treatment. Even if the nanoparticle successfully enters the bloodstream, it may have difficulty reaching all leukemia cells hidden within the bone marrow. Incomplete drug delivery could allow some cancer cells to survive and potentially cause the disease to relapse.

4. Nanoparticle Size Constraints
   The proposed nanoparticle is approximately 100–150 nanometers in diameter. Its size is important because it affects how well it circulates in the bloodstream and enters target cells. If the nanoparticle is too large, it may not efficiently reach its target. If it is too small, it may release the drug too quickly or be filtered out of the body. Finding the optimal size remains an important engineering challenge.

5. Manufacturing Challenges
   Producing targeted nanoparticles on a large scale is difficult and expensive. Scientists must ensure that every nanoparticle contains the correct amount of Cytarabine, has antibodies attached properly, and maintains a consistent size and structure. Manufacturing errors could affect the safety and effectiveness of the treatment.

Analysis

The CD33 targeted PLGA nanoparticle is scientifically established but would require extensive testing before clinical use. Its main advantages are the targeted delivery system, biodegradable materials, and the ability to carry Cytrabine directly to the CD33 positive AML cells. However, successful treatments would require the nanoparticles to overcome multiple barriers simultaneously. It must survive long enough in circulation to reach leukemia cells, and recognize CD33, avoid excessive bindings to healthy cells, and enter the target cell to release Cytarabine at an appropriate rate, and produce a sufficient therapeutic effect.

The biggest limitation of the current design is that targeting one receptor cannot guarantee that every AML cell will be reached or destroyed in the process. AML is heterogeneous, and CD33 is not completely cancer-specific, and the bone marrow microenvironment can protect leukemia cells from treatment. Therefore, the next stage of development would involve laboratory analysis and testing to determine the nanoparticle stability. If the experiments tested produce results that are promising, the design could then undergo increasingly rigorous preclinical trials/testing.

Ultimately, the purpose of this nanoparticle is not to eliminate every limitation of chemotherapy, but to investigate whether the drug delivery can be made more precise, controlled, and selective. The concept demonstrates how nanomedicine can combine molecular targeting, materials science, and pharmacology to address some of the challenges associated with AML treatments.

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Insights On the Role of Nanomedicines (ABRAXANE and Onivyde) in Pancreatic Cancer
By Erin P.Y. Chang

Abstract

Pancreatic cancer – a major public health and oncology focus over the years due to its late diagnostic stage, rapid progression, and limited response options. Its poor prognosis is correlated with its tumor microenvironment's ability to restrict drug penetration and increase therapeutic resistance. This review will examine pancreatic cancer by looking at new nanomedicine alternatives: ABRAXANE and Onivyde, to traditional conventional cancer treatments. These nanoparticle based systems can help improve drug solubility, prolong circulation in the system and support molecular targeting, which show promising development towards finding more effective treatment methods. Future progress will require the integration of nanoscale drug delivery with realistic 3D models, patient selective therapies, and improved manufacturing methods.

Keywords

Nab-paclitaxel, ABRAXANE, Pancreatic Cancer, Nanomedicines, Onivyde, Liposomal Irinotecan, Pancreatic Adenocarcinoma, Gemcitabine, NALIRIFOX

Introduction

The Pancreatic Adenocarcinoma – one of the deadliest and most lethal human malignancies, the third leading cause of death cancers, due to its frequent advanced stage diagnoses. Despite significant treatment efforts, this kind of cancer is not inherently "incurable", however it remains extremely challenging to cure because it is rarely detected before it has become aggressive and spread. While it is considered one of the deadliest cancers, it can still be cured if diagnosed in the early stages, although that opportunity is missed in over 80% of cases (Tonini and Zanni 2025). Nanomedicine is a technology which has helped provide the possibility of delivering drugs to specific cells.

Public health significance of cancers is determined by many factors such as survival rates, increasing or persistent diseases in populations, and diagnostic and treatment ability of current technology. Pancreatic cancer is significant because it is one of the leading causes of cancer death. Only a minority of cases are resectable at diagnosis, as it is usually identified after cancerous cells have spread and/or become locally advanced, making survival rates and outcomes poor.

Pancreatic cancer does not respond well to traditional treatments, especially chemotherapy. Its pathogenesis involves many factors including genetic mutations, environmental exposures, inflammation and abnormal tumor environments which further promote rapid growth and help with treatment resistance. This makes it difficult for therapeutic

methods to intervene with the cancer before late stages, resulting in pancreatic cancer remaining a large public health and oncology research priority.

Because of its highly aggressive malignancy, pancreatic cancer is highly fatal with low 5 year survival rates at about 12%. Pancreatic cancer can be divided into 2 larger categories: exocrine pancreatic cancer, which includes pancreatic ductal adenocarcinoma (PDAC), a disease more prevalent in the elderly; and neuroendocrine pancreatic cancer. The more common type: PDAC, develops in the ducts of the exocrine pancreas. Neuroendocrine pancreatic cancer starts in the endocrine cells of the pancreas. Biological behaviour of the disease is shaped by epithelial-stromal signaling, metabolic adaptation, and immunosuppression. Cancer that spreads from the pancreas to other parts of the body is called advanced pancreatic cancer, also known as metastatic or stage 4.

One of the defining features of pancreatic cancer is its tumor microenvironment, containing abundant extracellular matrixes, activated fibroblasts, poor vascularisation, and infiltrating immune cells. Pancreatic cancer progresses through microenvironmental changes that support invasion, metastasis, and immune evasion, it also develops through the accumulation of genetic alterations (John Hopkins Medicine, 2025), with common driver mutations including KRAS, TP53, CDKN2A, and SMAD4 (Arul et al 2022). This causes increased tissue stiffness, creating high interstitial pressure and reducing perfusion, restricting oxygen and drug penetration (Greene et al. 2021). Because of the stiffening, it reduces efficiency of chemotherapy due to poor delivery and drug resistance. Surgery is also not an option majority of the time, since fewer than 15% of people are diagnosed at a stage where resection is possible, and many patients recur (Ansari et al. 2015).

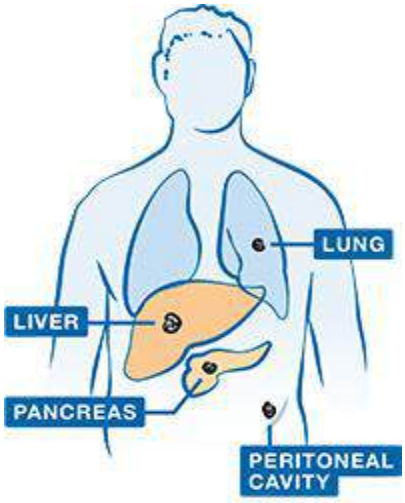


Figure 1: Diagram of Common Places Advanced Pancreatic Cancer Might Spread (BMS)

Common body parts of the cancer might spread to include the liver, the lung, and the peritoneal cavity (Figure 1). Risk factors for pancreatic cancer include older African American men, obesity, lifestyles that include heavy tobacco use, exposure to certain chemicals, and family

history which contribute from 5-10% of known cases. It is important to note that people can still get pancreatic cancer even if they don't have any risk factors (Viegas et al, 2023).

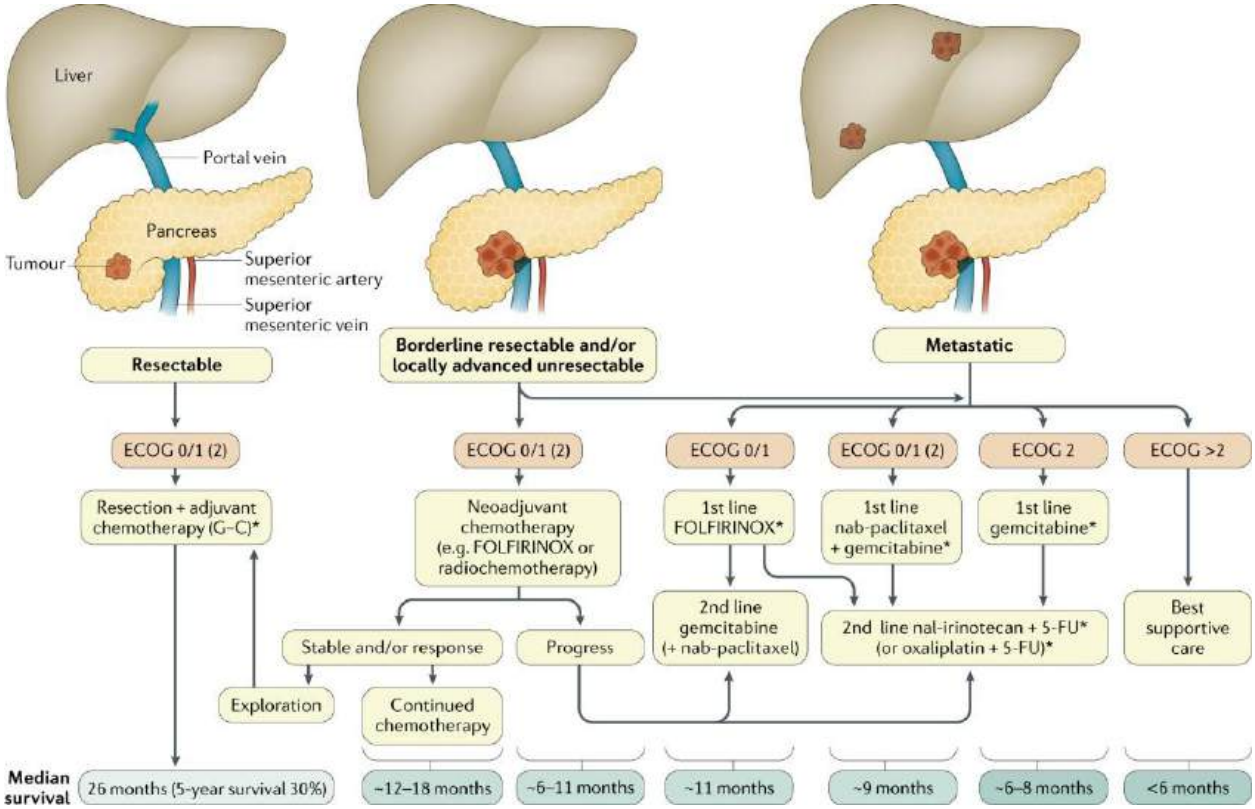


Figure 2: Possible Treatments for Pancreatic Cancer (Neoptolemos 2018)

Despite advances in oncology, due to its poor prognosis, high mortality rates, and resistance to standard cancer treatments such as surgery, chemotherapy, radiation therapy, and targeted therapy (Figure 2), this cancer still poses major public health challenges. These traditional approaches have limited effectiveness in pancreatic cancer because of the tumor's dense microenvironment, which makes drug penetration difficult, contributing to treatment resistance. Overall looking at all stages combined, there is a 13% of five year relative survival (Elshami et al. 2026)

One area of development is nanomedicine, which can possibly be used as an alternative treatment to cancer. Nanomedicine is the application of nanotechnology, often using small materials, devices or systems in nanometers to treat, prevent, and monitor diseases. Nanoscale drug carriers have been evolving to improve how anticancer medicines are delivered into the body.

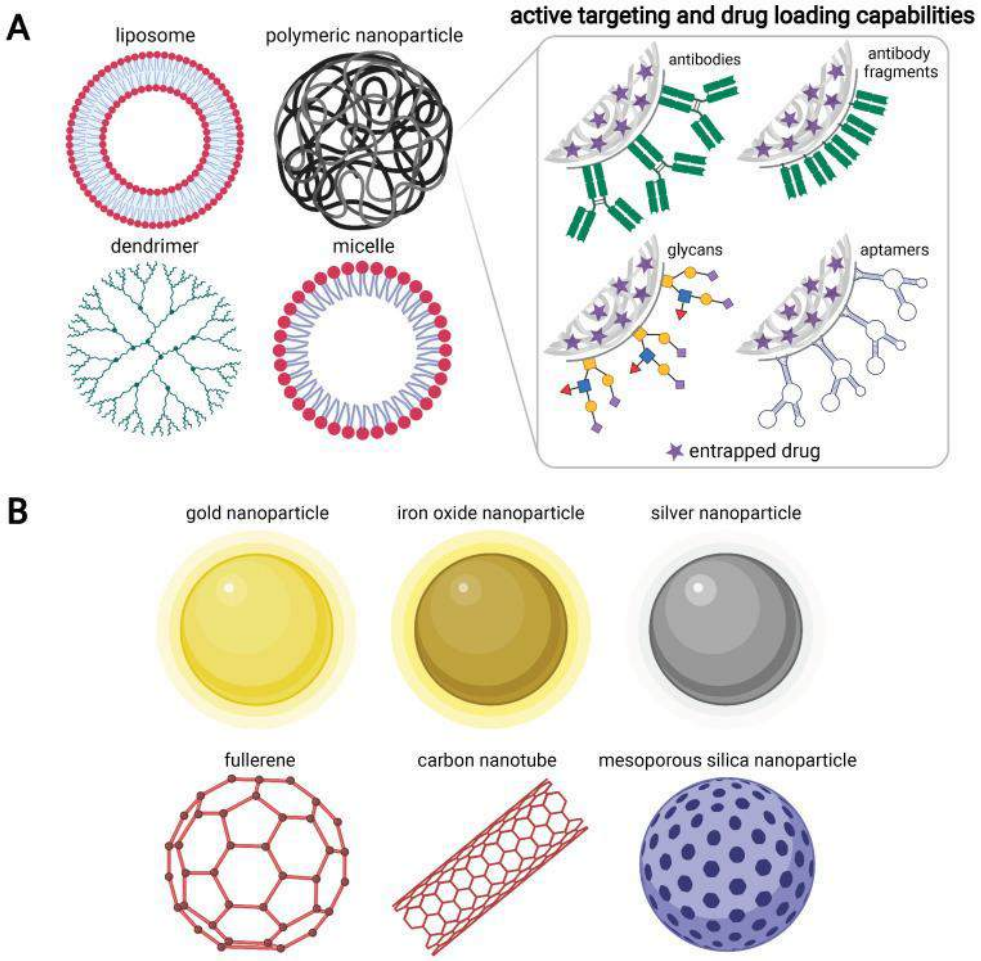


Figure 3: Types of Nanodrug Carriers (Greene et al. 2021)

The biotechnology of these drug carriers now include liposomes, gold, silver, iron oxide nano particles, fullerene, etc. They can bypass the dense scar tissue surrounding pancreatic cancer. Compared to traditional methods, nanomedicine can enhance drug stability, improve penetration into solid tumors and support targeted delivery to areas with cancer cells while sparing healthy tissue. This makes nanomedicine relevant to pancreatic cancer, especially as poor drug delivery is a major issue stopping healthcare professionals from administering successful treatment. It is currently being developed by research groups to help address weaknesses of traditional cancer therapy methods, including low specificity, side effects and limited tumor accumulation. Some nanomedicines are currently being designed to target both cancer cells and surrounding tumor microenvironment such as cancer-associated fibroblasts and extracellular matrix components, to improve treatment penetration and effectiveness. Newer nanotechnology also combines multiple therapeutic strategies, such as drug delivery, tumor targeting, therapy enhancement and biodistribution. This shows potential for personalized and adaptive cancer treatment (Viegas et al. 2023).

Nanomedicine has already shown its importance and effectiveness in treatment processes of other cancers. For example, nanomedicine has been widely used in breast cancer treatment, such as clinical products like DOXIL, used for advanced or metastatic breast cancer. This demonstrates that nano-based drug delivery can improve therapeutic outcomes, supporting continued interest and success in applying more nanomedicine in cancers.

With limitations of the pancreas environment, successful nanomedicine engineering regarding drug transport requires a combination: reduction and navigation of stromal barriers, then delivering therapeutic agents to both tumor and stromal cells, and finally the ability to monitor whether the nanoparticles reach the intended tissue. These qualities are important because improving drug potency alone may not be able to solve dense microenvironment issues that cause treatment failure.

According to Monteiro et al., it is difficult to deal with pancreatic cancer due to its non-uniform-like target. The microenvironment makes standard 2D cell cultures inadequate for predicting therapeutic response. Animal models are generally limited to capturing heterogenous and fibrotic stroma components of PDAC, unable to fully capture the complexities of human cancerous tumor microenvironments, limiting testing and explaining why many treatments don't completely succeed clinically (Monteiro et al. 2022).

Important priorities of biomedical engineering of nanotechnology include realistic disease models, therapeutic systems, drug carriers, biodegradable materials, reproducible manufacturing, and patient specific targeting for pancreatic cancer.

This review will examine the two commercialised nanomedicines for the treatment of pancreatic cancer – ABARAXANE and Onivyde – exploring their efficacies and limitations.

Nanomedicines

ABRAXANE

ABRAXANE, also known as Nab-paclitaxel, is one of the around 80nm (Bobo et al. 2016) that have been FDA approved. ABRAXANE was developed by Dr. Patrick Soon-Shiong, a surgeon and biotechnology entrepreneur from South Africa and America, with Chemical Engineer Dr. Niel Desai (Los Angeles Business Journal 2017). The nanomedicine was invented at Los Angeles based biotechnology firm VivoRx. It can be used for treatment of metastatic breast cancer, locally advanced or metastatic lung cancer, and advanced pancreatic cancer, with a combination of other traditional medicines. When used in combination with gemcitabine, it is the first medicine you receive to treat advanced pancreatic cancer. It is a form of chemotherapy, used to kill or prevent growth of cancer cells. Treatment with this medicine can help to control or slow the spread of cancer cells.

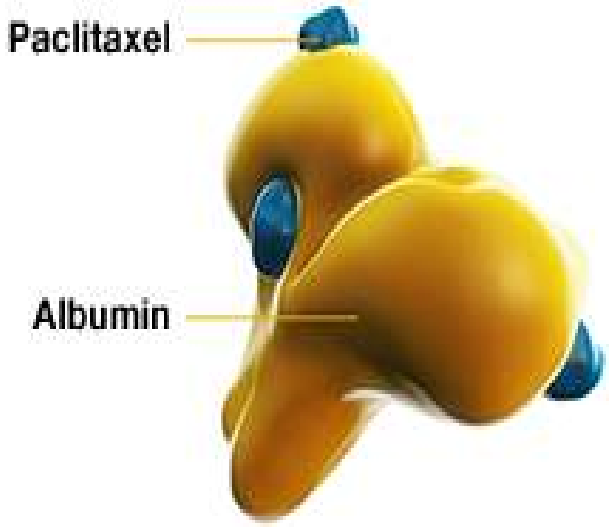


Figure 4: ABRAXANE Nanomedicine Structure (BMS)

ABRAXANE is composed of cancer fighting medicine paclitaxel bound to albumin, a protein found in blood. Paclitaxel, traditionally dissolved in a solvent, is the active drug that makes ABRAXANE a nanomedicine. It is packaged as very small albumin bound nanoparticles, about 130nm (Figure 4). The albumin carrier is given intravenously and helps the drug move through the bloodstream and into tumors more effectively than solvent-based paclitaxel. This is a form of targeted delivery rather than improved target delivery and tumor uptake. Though because ABRAXANE does not contain any solvents, it is not usually necessary to take medicines that prevent allergic reactions, yet allergic reactions can still occur when taking this nanomedicine. ABRAXANE enters tumors through albumin related transport mechanisms, including but not limited to gp60 (albumin receptors) mediated transcytosis, and SPARC-related binding in the tumor microenvironment. Gp60 mediated transcytosis is a mechanism where albumin binds to receptors on the blood vessel lining cells and is transported across into the tumor area. SPARC related binding is another mechanism. SPARC is a protein receptor found in some tumors that may bind albumin and help retain the drug in cancerous tissue. While SPARC is still undergoing studies and active assembly, it is known that gp60 and SPARC have important roles in drug delivery.

ABRAXANE works by preventing cancerous cell division. Action proteins called microtubules are interfered with by paclitaxel as it binds to tubulin and stabilizes them. This prevents the cell from completing mitosis, causing them to eventually die (Figure 5). The drug travels efficiently through the bloodstream through its albumin based nanoparticle structure, and accumulates in tumors. This makes the ABRAXANE work effectively against pancreatic cancer because the tumor microenvironment is usually dense and difficult for traditional drugs to penetrate.

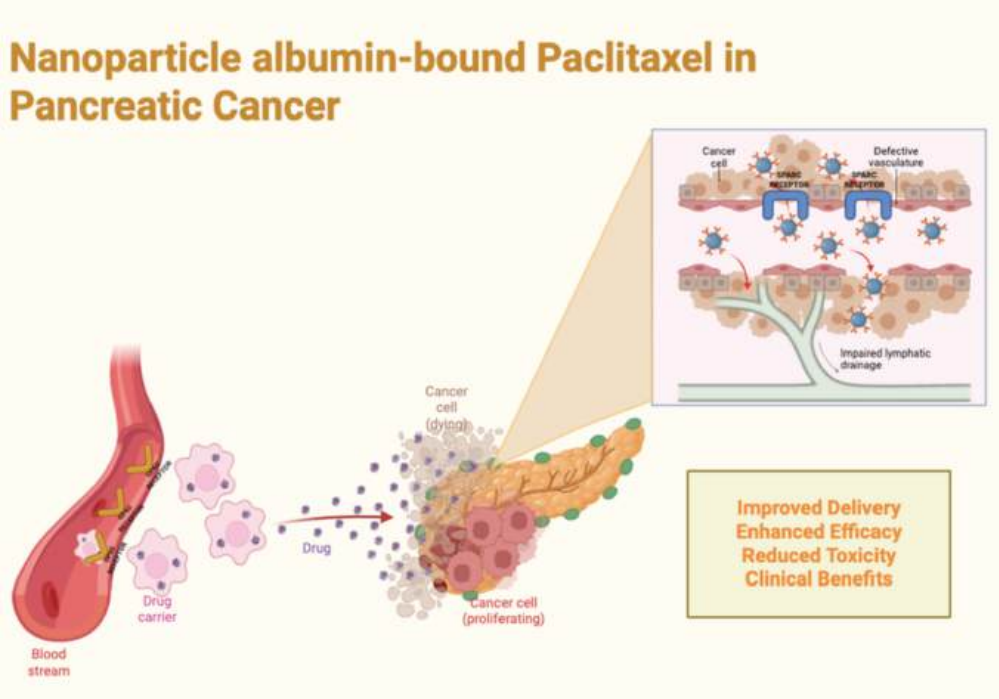


Figure 5: Nanoparticle Albumin-bound Paclitaxel in Pancreatic Cancer: Mechanisms and Advantages (Konstantinidis et al. 2025)

ABRAXANE is often given in combination with gemcitabine, as the pairing has shown to improve survival rates compared to just gemcitabine alone (Table 1)(Von Hoff et al. 2013) (Al-Hajeili et al. 2014).

Table 1: Data Comparison of ABRAXANE + Gemcitabine Vs. Gemcitabine Monotherapy (Von Hoff et al. 2013) (Al-Hajeili et al. 2014)

	ABRAXANE + Gemcitabine	Gemcitabine monotherapy
Median Overall Survival	8.5 months	6.7 months
1-year survival	35%	22%
2-year survival	9%	4%
Median progression free survival	5.5 months	3.7 months
Objective response rate	23%	7%

Gemcitabine is a drug that damages cancer cells, and it does so by interfering with DNA synthesis. Since tumors in pancreatic cancer are hard for drugs to penetrate, ABRAXANE improves treatment by improving delivery of drugs into tumors. Though the combination is a stronger option than gemcitabine or paclitaxel alone, it is still not a cure and is mainly used to control the growth of cancerous cells (Ciccolini et al. 2016).

Compared to traditional chemotherapy, ABRAXANE has several advantages. Paclitaxel on its own requires solvents such as cremophor and ethanol to deliver the medicine, which can be linked to toxicity and other formulation problems; ABRAXANE has been designed to avoid those issues.

ABRAXANE is a form of chemotherapy, so it also causes chemotherapy-like side effects. However, it can also cause additional complications, including neutropenia, peripheral neuropathy, fatigue, nausea, diarrhea, and low platelet counts. Peripheral neuropathy can be considered one of the more notable repercussions, though it is often reversible when treatment is paused or the dosage has been decreased. Those who should not take Abraxane are people with reduced white blood cell counts – below 1500 cells/mm^3 – because of reduced bone marrow activity which can increase chances of getting a serious infection, or ones who have had severe allergic reactions to ABRAXANE. ABRAXANE dosing is based on approved regimens and toxicity monitoring (Bristol Myers Squibb 2013).

ABRAXANE is generally more expensive than standard chemotherapy due to it being a branded nanomedicine product, and part of a specialised treatment regimen. It costs about $1550USD per 100mg vial (Drugs.com 2026), compared to chemotherapy which costs about $27000USD over a 6 month course (Carrera PM et al. 2018). Its cost efficiency depends mainly on the health system and the survival benefits compared to its price on different people and in different situations.

Onivyde

Onivyde, also known as liposomal irinotecan, was FDA approved in 2015 for firstline treatment of metastatic pancreatic adenocarcinoma. It is the nanomedicine formulation of a chemotherapy drug – irinotecan – enclosed by liposome drug carriers. It is often given intravenously in combination with oxaliplatin, fluorouracil and leucovorin, a mixture otherwise known as NALIRIFOX. In phase III clinical trials, NALIRIFOX has shown significantly improved overall survival and progression free survival compared to ABRAXANE used with gemcitabine when used as first-line treatment of patients with metastatic pancreatic ductal adenocarcinoma (Brown, Blair 2025).

Liposomal irinotecan (Figure 6), 110nm wide in diameter, works as an inhibitor for the topoisomerase I (European Medicines Agency, 2016). Onivyde is irinotecan packaged inside a tiny lipid shell called liposome, instead of being given as a free drug. Irinotecan is trapped inside the inner compartment of the liposome using sucrose octasulfate as a trapping agent. This protects the drug from premature breakdown, while prolonging circulation time and increases drug exposure time in the tumor (Figure 7). It controls where and when irinotecan is released so the drug is generated more effectively near the tumor.

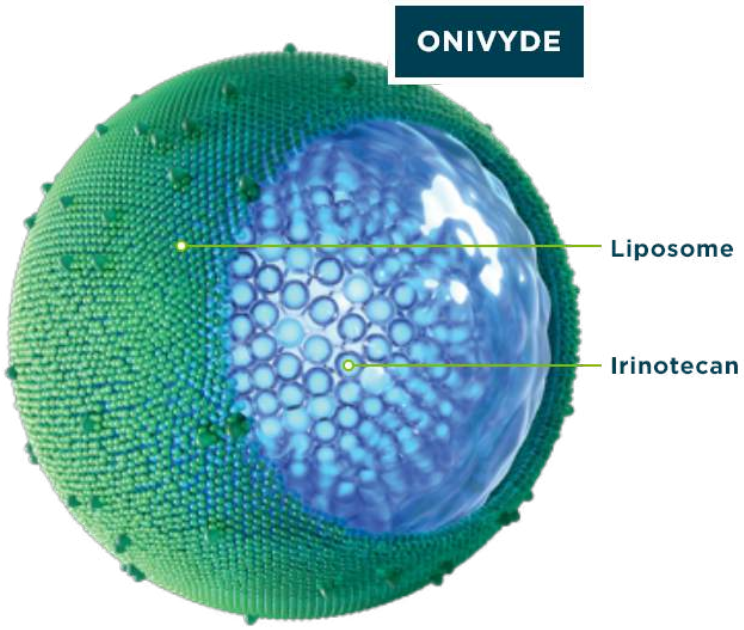


Figure 6: Onivyde Nanomedicine Structure

Given by IV infusion, Onivyde travels through the bloodstream instead of being absorbed in the gut. Irinotecan is firstly converted to its active metabolite SN-38, which is more cytotoxic than irinotecan and is able to block topoisomerase I. That is an enzyme cancer cells need to relieve DNA twisting during replication. The enzyme being unable to activate causes DNA damage, replication failure, and apoptosis. Liposomal irinotecan is not a single agent and is not interchangeable with conventional non-liposomal irinotecan (Brown, Blair 2025).

Patients would be premedicated with corticosteroid such as dexamethasone with an anti-emetic more than or equal to 30 minutes before treatment with liposomal irinotecan. NALIRIFOX is administered as a sequential intravenous infusion of liposomal irinotecan, oxaliplatin, leucovorin, and fluorouracil. This regimen is given to patients every 2 weeks until disease progression is seen or unacceptable toxicity. Because pancreatic cancer is highly resistant and one drug alone is often not enough, combination therapy is used. For Onivyde, regulatory safety review reports a lethal dose of 200mg/kg in mice, and 80mg/m^2 for humans (Brown, Blair 2025). The nanomedicine formulation of irinotecan is better at maintaining drug concentrations in the bloodstream and delivering the medicine to tumours. NALIRIFOX showed improved response rates during clinical trial stages, compared to other second line regimens and traditional irinotecan. Complications include neutropenia, diarrhea, vomiting, anemia, fatigue and reduced appetite. The nano-formulation does help improve treatment delivery and can make the drug more effective with lower exposure to healthy tissue even with these side effects, compared to traditional therapeutic methods (Ipsen, 2025).

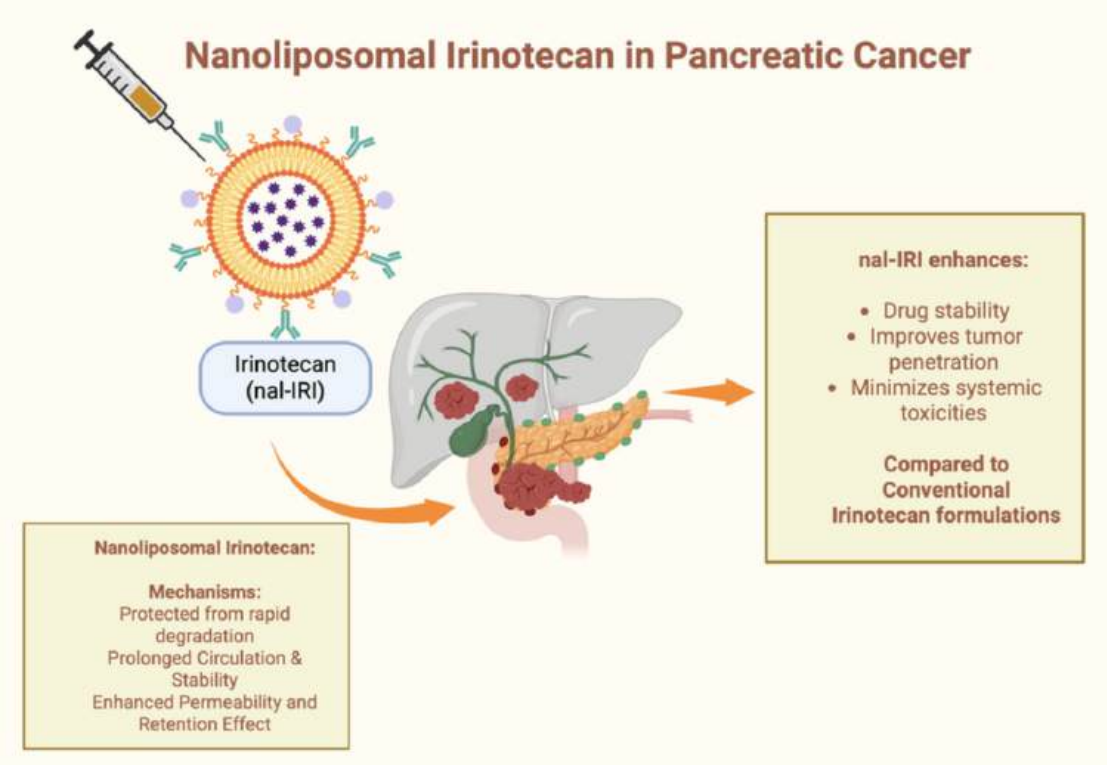


Figure 7: Nanoliposomal Irinotecan in Pancreatic Cancer: Mechanisms and Advantages (Konstantinidis et al. 2025)

Though with its improved medicinal properties, it also comes with side effects. NALIRIFOX can cause severe and life threatening neutropenia and diarrhea. To patients who have experienced severe hypersensitivity reaction or anaphylaxis to Onivyde or irinotecan HCL, Onivyde is contraindicated. It is also important to note that there has been permanent discontinuation of Onivyde in 17% of patients due to adverse reactions (Ipsen 2025).

In terms of cost, liposomal irinotecan costs about $3000USD per supply of 10mL, and you use 2-4 vials per infusion, given every 2 weeks (Drugs.com 2026). Combined, it is often more expensive than traditional chemotherapy due to it being, like ABRAXANE, a specialised branded nanomedicine, used in complex combination regimens. Comparing nanomedicines ABRAXANE and Onyvide, it is hard to make direct comparisons due to the fact that they contain different drugs and are used in different regimens. Yet recent evidence of nanomedicine used for metastatic pancreatic cancer favors Liposomal irinotecan based NALIRIFOX rather than ABRAXANE combined with gemcitabine. In the NAPOLI-1 trial, NALIRIFOX improved overall survival and progression free survival compared with ABRAXANE in treatment of naive metastatic pancreatic ductal adenocarcinoma. However, ABRAXANE is already a widely recognised established nanomedicine for pancreatic cancer as opposed to NALIRIFOX, and is often simpler as the mechanism is straightforward. This makes ABRAXANE, especially when paclitaxel based chemotherapy is preferred, remain the standard option.

Conclusion

Both nanomedicines ABRAXANE and Onivyde are nanomedicines that can improve drug delivery into the dense pancreatic tumor microenvironment. They are alternatives to traditional methods of treating cancer such as chemotherapy and radiation therapy.

ABRAXANE benefits from albumin-mediated transport with gp60 transcytosis and SPARC binding, to concentrate paclitaxel in tumors. When combined with gemcitabine, it delivers a survival advantage over gemcitabine alone. Onivyde encapsulates irinotecan in liposomes to prolong circulation and enhance tumor exposure of active metabolite SN-38. Used in the NALIRIFOX regimen, data indicate improved overall progression-free survival compared with ABRAXANE + gemcitabine in treatment of the metastatic disease, supporting its role as a newly emerging first-line option.

Table 2: Comparisons between ABRAXANE with gemcitabine and Onivyde in NALIRIFOX

	Delivery	Price (USD)	Cautions	Toxicities	Public Health Positioning
ABRAXANE + gemcitabine	Solvent-free, albumin-mediated tumor uptake	~$1550 per 100mg vial	Baseline neutrophils <1500 cells/mm^3, history of severe hypersensitivity to ABRAXANE	Neutropenia, peripheral neuropathy, fatigue, nausea, diarrhea, thrombocytopenia	Established, broadly implementable as first line treatment
Onivyde in NALIRIFOX	Liposomal encapsulation prolongs circulation in body, protects drug and enhances tumor exposure with controlled release	~$3000 per 10mL vial (2-4 vials per infusion)	Severe hypersensitivity/anaphylaxis to Onivyde or irinotecan	Neutropenia (severe/life threatening), diarrhea, vomiting, anemia, fatigue, reduced appetite	Higher efficacy signals in fit patients, less established, more resources and infusion capacity required to support complex regimens

Making a weighted public health judgement by prioritising survival benefit, manageability, access and implementation feasibility shows that NALIRIFOX can be favored when resources and infusion capacity allow. This is given its better first line efficacy signal, however ABRAXANE treated with gemcitabine still remains the more universally implementable and accessible standard, due to its longer track record, simpler logistics and larger familiarity. While for population impact today, ABRAXANE and gemcitabine offer better value and are more cost efficient, for clinics or hospitals that are able to manage higher toxicity and complex infusions, NALIRIFOX provides greater chances for survival and should be preferred when accessible. Conventional chemotherapy and treatments will continue to hold fundamental roles in cancer therapy, but nanomedicines still have lots of discoveries to be made, reducing side effects and increasing survival rate.

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Association Between Breastfeeding History and Breast Cancer Occurrence: Findings from a Survey-Based Study By Siffet Gondara

Abstract

Breast cancer is the most common type of cancer among women in the United States, accounting for approximately 30% of all new female cancer diagnoses each year, with an estimated 321,910 new invasive cases and 42,140 deaths projected for 2026 alone. Incidence rates have risen by 1% annually in recent years, highlighting the urgency of identifying modifiable risk factors (1). Established risk factors include genetic mutations (such as BRCA1 and BRCA2), reproductive history, dense breast tissue, and lifestyle factors such as physical inactivity, excess body weight, and alcohol consumption. (3) While prior research has established an association between breastfeeding and reduced breast cancer risk, insufficient attention has been given to the role of breastfeeding duration in shaping this relationship. To address this gap, an anonymous online survey was distributed across the United States to women previously diagnosed with breast cancer, collecting data on breastfeeding history, duration, and demographic characteristics. Analysis of the collected data revealed that longer breastfeeding duration is associated with potential protective benefits against breast cancer, contributing to the existing literature and supporting more informed health decisions among women.

Introduction

Nearly 1 in 8 women in the United States will be diagnosed with breast cancer in their lifetime, making it one of the most common and devastating diseases affecting women today. (11). With thousands of new cases diagnosed worldwide each year, considerable research has focused on identifying risk factors and protective factors to better understand the disease and determine potential prevention strategies (8). Breast cancer develops through the accumulation of DNA mutations that cause uncontrolled growth of breast tissue cells, forming a tumor that can invade surrounding tissue or metastasize to other parts of the body (6). Breast cancer is not a single uniform disease but rather includes distinct biological subtypes, classified based on the presence or absence of hormone receptors and HER2 protein expression, including HR+/HER2−, HR+/HER2+, HR−/HER2+, and triple-negative breast cancer (TNBC) (5). These subtypes differ in their behavior, prognosis, and response to treatment, making subtype classification an important aspect of understanding disease risk and progression (7). Among the most well-known mutations linked to breast cancer are those in the BRCA1 and BRCA2 genes, which normally help the body repair damaged DNA; when mutated, they can interfere with this repair process and lead to abnormal cell growth (9). Beyond BRCA1 and BRCA2, mutations in at least 11 other genes, including PALB2, ATM, TP53, CHEK2, and PTEN, have also been identified as increasing breast cancer susceptibility (9). These mutations can be inherited or triggered by a range of influences, including aging, hormonal exposure, radiation, and certain lifestyle factors (10). Certain lifestyle behaviors, including regular physical activity and maintaining a healthy

body weight, have been associated with a reduced risk of breast cancer (2). Reproductive and maternal health practices may also play a meaningful role in disease risk. Breastfeeding, in particular, has been proposed to offer a protective effect through mechanisms such as reduced lifetime estrogen exposure and the promotion of breast tissue differentiation (4). Despite growing evidence supporting this association, the role of breastfeeding duration in shaping breast cancer risk remains insufficiently studied, representing an important gap this research seeks to address.

The purpose of this study was to explore the relationship between breast cancer risk and breastfeeding. Specifically, this research investigated whether breast cancer risk is correlated with the duration of breastfeeding. Survey data were collected from women who had been diagnosed with breast cancer to evaluate the potential correlation between breastfeeding history and disease risk. By improving understanding of this relationship, the findings from this study may promote greater awareness of breastfeeding as a potential factor in breast cancer prevention and support informed health decisions by increasing global awareness among women.

Methods

This study utilized an online survey (Google Forms) to collect data from women who had been diagnosed with breast cancer or were currently living with the disease. Participants were recruited through hospitals, health centers, and breast cancer support groups, which distributed the survey link to eligible patients and members. Upon agreeing to participate via informed consent, individuals completed the survey electronically. The survey required approximately 5 minutes to complete and included information regarding participants' demographic characteristics, breast cancer history, and breastfeeding practices.

The study aimed to collect approximately 100 responses for analysis. Following collection, the data were compiled and analyzed to identify potential trends and correlations between breastfeeding and breast cancer risk. All collected data were stored securely and maintained in a confidential manner to protect participants' privacy.

Results

Participants' demographics and statistics

Table 1 presents the demographic characteristics of the 33 participants included in this study. The majority of participants were between the ages of 41 and 50 (48.5%), followed by those aged 31 to 40 (27.2%), 51 to 60 (18.2%), 20 to 30 (3.0%), and 61 to 70 (3.0%). It should be noted that ages reported in this study represent the participants' age at the time of the survey completion and do not reflect their age at breast cancer diagnosis or their age during breastfeeding. This age distribution is heavily concentrated in middle-aged participants, and data may not be normally distributed. Future studies should consider collecting age at first pregnancy, as this factor may provide additional insight into the relationship between reproductive history and breast cancer risk. Regarding racial composition, the sample was predominantly Caucasian

(72.7%), with smaller representations of Black/African American (9.0%), Asian (9.0%), and other racial groups (9.0%). No Hispanic/Latino participants were included in this sample. This racial imbalance represents a notable limitation, as findings may not be fully generalizable across racially diverse populations. Future studies should aim to recruit a more racially diverse participant pool to strengthen the validity of the results. Beyond breastfeeding behaviors, several lifestyle factors known to influence breast cancer risk were also collected, including physical activity levels, dietary habits, alcohol consumption, and smoking history. For this study, these variables were designated as non-contributing factors, as the primary focus was on the relationship between breastfeeding duration and breast cancer risk. However, future research should consider incorporating and controlling for these variables to better isolate the effect of breastfeeding and minimize potential confounding bias.

Table 1. Demographic Characteristics of Participants.

Demographic	Characteristics	Number of participants (n)	Percentage (%)
Age	Age 20-30	1	3
	Age 31-40	9	27.2
	Age 41-50	16	48.5
	Age 51-60	6	18.2
	Age 61-70	1	3
Race/ethnicity	Caucasian	24	72.7
	Black/African American	3	9
	Hispanic/Latino	0	0
	Asian	3	9
	Not Reported	3	9
Number of Children	0 Children	3	9
	1 Child	6	18.2
	2 Children	13	39.4
	3 Children	7	21.2
	4+ Children	4	12.1

Breastfeeding duration was generally longer among patients with HR+/HER2– breast cancer, whereas HER2+ and triple-negative subtypes showed more variable breastfeeding patterns with no clear directional association.

Among participants with a family history of breast cancer

Analysis of participants with a family history of breast cancer revealed notable differences in breastfeeding duration across subtypes **(Table 2)**. HR+/HER2− cases in this group demonstrated moderate to longer breastfeeding durations; Patient 5 breastfed four children for a combined total of approximately six years, Patient 11 breastfed two children for 12 months each, and Patient 19 breastfed three children for 10, 8, and 6 months, respectively. In contrast, TNBC cases showed more varied patterns: Patient 9 breastfed for only 10 months total, and Patient 21 breastfed for just 6 months, while Patient 25 breastfed three children for a combined total of five years. HER2+ cases such as Patient 4 breastfed two children for 9 and 7 months, while Patient 23 breastfed two children for 2.5 and 4.5 years, respectively, representing an exception to the general pattern. These findings suggest that among women with a family history, HR+/HER2− subtypes were more commonly associated with moderate breastfeeding durations, though exceptions exist. In terms of subtype distribution within this group (n = 15), TNBC accounted for 26.7% (4/15), HR+/HER2− for 20.0% (3/15), HER2+ for 13.3% (2/15), and a mixed HR+/HER2+ profile for 6.7% (1/15), with subtype not reported for the remaining 33.3% (5/15) **(Figure 1 (A)).**

Among participants without a family history of breast cancer

Among participants without a family history of breast cancer, HR+/HER2− cases were associated with notably longer breastfeeding durations. Patient 14 breastfed two children for over two years each, Patient 15 breastfed for two full years, and Patient 33 breastfed five children with durations ranging from 15 to 24 months per child. In contrast, HER2+ cases such as Patient 2 breastfed three children for 10, 6, and 6 months, respectively, reflecting shorter overall durations. TNBC cases in this group showed limited data, with Patient 26 having no recorded breastfeeding data. Triple-positive cases such as Patient 22 breastfed two children for 2.5 and 4.5 years, representing longer durations within a more aggressive subtype. Overall, these patterns support the claim that longer breastfeeding duration was more commonly observed among HR+/HER2− cases in women without a family history, while shorter durations were more prevalent among HER2+ subtypes. In terms of subtype distribution within this group (n = 16), HR+/HER2− was the most common subtype at 25.0% (4/16), followed by triple-positive disease at 18.75% (3/16), HER2+ and HR+ (HER2 status unspecified) each at 12.5% (2/16), and TNBC at 6.25% (1/16), with subtype not reported for 25.0% (4/16) **(Figure 1 (B))**.

Table 2. Patient Biometrics

Family History of Breast Cancer	Patient ID	Age at Diagnosis	Ethnicity	Subtype
Yes	4	46	White	HER 2 positive
Yes	5	52	White	ER+/ PR- Her 2 -

Yes	6	50	White	
Yes	9	32	White	Triple Negative (TNBC)
Yes	11	44	White	Hormone receptor–positive / HER2–negative (Luminal A–type)
Yes	16	45	White	
Yes	17	38	White	HR+/HER2+
Yes	18	40		Triple-Negative (TNBC)
Yes	19	48	White	HR/progesterone+ HER -
Yes	21	46	White	HER2– / ERPR+, TNBC
Yes	23	42	American Indian or Alaska Native, Black or African American, White	HER2-positive
Yes	25	47	White	Triple Negative (TNBC)
Yes	28	51	Asian	
Yes	30	50	White	
Yes	31	44		
No	1	38	White	ER/PR+, HER2-
No	2	37	White	HER2+
No	3	38	White	Hormone receptor–positive (HR+)
No	7	30	White	Triple Positive (ER+/PR+/HER2+)
No	8	30	White	
No	10	41	White	
No	12	38	White	Triple positive
No	13		White	
No	14	37	White	HR+/HER2– (Luminal A or B)
No	15	35	White	HR+/HER2– (Luminal A–type).
No	22	32	Black or African	Triple Positive (ER+/PR+/HER2+)

			American	
No	24	45	Asian	
No	26	31	African	Triple Negative (TNBC)
No	27	49	White	ER+/PR+
No	32	43	South African Black	HER2-positive
No	33	55	White	HR+/HER2–
Uncertain	20		White	
Uncertain	29	51	Asian	ER2-positive

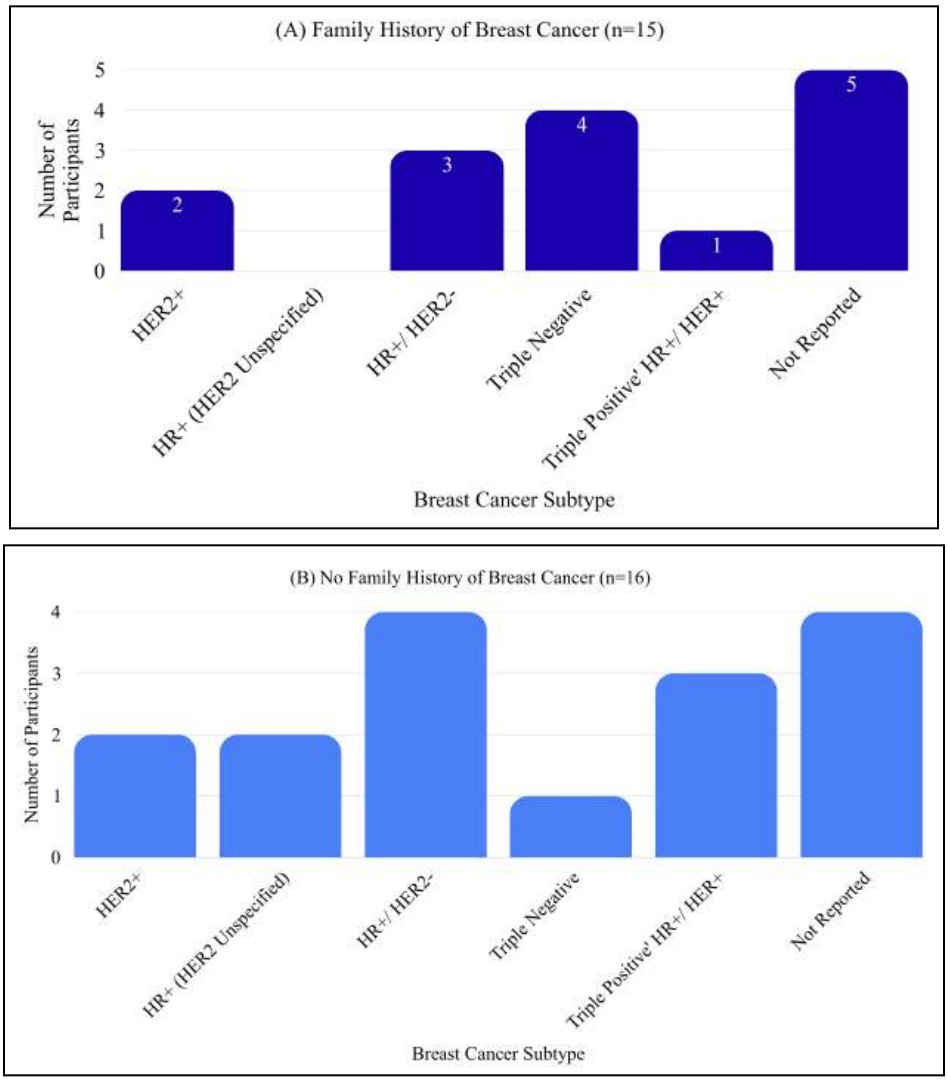


Figure 1. Breastfeeding distribution by subtype. (A) Among participants with a family history of breast cancer. (B) Among participants with no family history of breast cancer.

Diagnoses clustered into two distinct age patterns: triple-positive and HR+/HER2+ subtypes were diagnosed almost exclusively before age 40, while HER2+ and ER+/PR+/HER2− subtypes were more often diagnosed after age 40.

Among triple-positive (ER+/PR+/HER2+) patients (n = 3), 33.3% (1/3) were diagnosed between ages 20 to 30 and 66.7% (2/3) between ages 31 to 40, with no cases diagnosed after age 40. For example, Patient 12, diagnosed at age 38 with triple-positive breast cancer, had breastfed two children for 3 to 6 months. HR+/HER2+ patients (n = 2) showed a similarly young pattern, with both cases (100%) diagnosed between ages 31 to 40. In contrast, HER2+ patients (n = 5) were most often diagnosed after age 40, with 60% (3/5) diagnosed between ages 41 to 50 and 20% (1/5) between ages 51 to 60. Together, 80% (4/5) of HER2+ cases occurred after age 40, compared with only 20% (1/5) between ages 31 to 40. ER+/PR+/HER2− patients (n = 9) followed a similar though less pronounced pattern: 44.4% (4/9) were diagnosed between ages 41 to 50 and 22.2% (2/9) between ages 51 to 60, meaning 66.7% (6/9) of cases occurred after age 40, versus 33.3% (3/9) diagnosed between ages 31 to 40. TNBC cases (n = 5) fell largely between these two patterns, with 60% (3/5) diagnosed between ages 31 to 40 and 40% (2/5) between ages 41 to 50, and no cases before age 31 or after age 50. Taken together, these findings support a clear difference in age at diagnosis: triple-positive and HR+/HER2+ disease were confined to younger participants (age 40 or under), while HER2+ and ER+/PR+/HER2− disease were more frequently diagnosed after age 40. This pattern was most pronounced in HER2+ cases, where 80% of diagnoses occurred past this threshold **(Table 3).**

Table 3. Age of diagnosis for each cancer subtype.

Breast Cancer Subtype	20-30 years n (%)	31-40 years n (%)	41-50 years n (%)	51-60 years n (%)	Total
HER2+	0 (0%)	1 (20%)	3 (60%)	1 (20%)	5
ER+/PR+, HER2−	0 (0%)	3 (33.3%)	4 (44.4%)	2 (22.2%)	9
HR+/HER2+	0 (0%)	2 (100%)	0 (0%)	0 (0%)	2
Triple Positive (ER+/PR+/HER2+)	1 (33.3%)	2 (66.7%)	0 (0%)	0 (0%)	3
Triple Negative (TNBC)	0 (0%)	3 (60%)	2 (40%)	0 (0%)	5

Discussion

The findings of this study contribute to the growing body of literature suggesting that breastfeeding duration may play a protective role against breast cancer, while also highlighting how this relationship differs across molecular subtypes. Among participants with HR+/HER2− disease, breastfeeding durations were generally longer, both in women with and without a family

history of breast cancer. This pattern held whether breastfeeding was measured per child or summed across multiple children, and it was one of the more consistent trends observed across the dataset. This aligns with the proposed biological mechanism by which breastfeeding reduces lifetime estrogen exposure and promotes terminal differentiation of breast tissue, a process thought to lower the number of cells susceptible to hormone-driven malignant transformation (4). Because HR+/HER2− tumors are hormone-receptor driven, a subtype whose growth is fueled by estrogen exposure would be expected to be the subtype most sensitive to a protective factor that works by lowering lifetime estrogen exposure, which is consistent with what was observed here.

By contrast, breastfeeding patterns among HER2+ and triple-negative participants were less consistent, with several individuals in these groups reporting shorter breastfeeding durations and a few notable exceptions reporting longer durations. This variability may reflect the underlying biology of these subtypes: HER2+ and triple-negative tumors are not primarily driven by hormone receptor signaling, so a hormonally mediated protective mechanism such as breastfeeding would plausibly have a weaker or less predictable relationship with these subtypes. This distinction may help explain why prior studies examining breastfeeding and breast cancer risk in aggregate, without stratifying by subtype, have produced mixed or inconclusive results; pooling hormone-sensitive and hormone-insensitive subtypes together could dilute a protective association that is more clearly present within HR+/HER2− disease specifically.

The study also identified a secondary pattern relating age at diagnosis to subtype. Triple-positive and HR+/HER2+ cases were diagnosed almost exclusively before age 40, while HER2+ and HR+/HER2− cases were more often diagnosed after age 40. While this study was not designed to test causal explanations for this pattern, it is consistent with existing literature indicating that certain aggressive, hormone-and-HER2-co-driven subtypes tend to present in younger patients, whereas HER2+-only and luminal subtypes more often present later in life (7). This finding underscores that age at diagnosis, like breastfeeding history, may need to be considered in a subtype-specific rather than a generalized manner in future breast cancer risk research.

Several limitations should be considered when interpreting these results. First, the sample size was small (n = 33), and participants were predominantly middle-aged and Caucasian, which limits the generalizability of these findings to more racially and demographically diverse populations. Second, participants reported their current age rather than their age at diagnosis or the age at which breastfeeding occurred, and subtype and breastfeeding data were missing for a portion of participants, which may have introduced bias into the subtype-specific comparisons. Third, this study did not control for other lifestyle factors known to influence breast cancer risk, such as physical activity, diet, alcohol use, and smoking history, even though these variables were collected; future analyses that adjust for these factors would help isolate the independent contribution of breastfeeding duration. Fourth, because participants were recruited exclusively from individuals already diagnosed with breast cancer, this study cannot directly compare breastfeeding patterns to those of a cancer-free control group, which limits the ability to draw

firm conclusions about relative risk reduction. Finally, as a retrospective, self-reported survey, the data are subject to recall bias, particularly for breastfeeding durations that may have occurred many years prior to survey completion.

Future research should aim to recruit a larger and more racially diverse sample, include a cancer-free comparison group, and collect additional reproductive variables such as age at first pregnancy and total lifetime breastfeeding duration across all pregnancies. Statistically controlling for lifestyle covariates would also strengthen confidence that any observed associations are attributable to breastfeeding rather than to other correlated behaviors. Stratifying analyses by molecular subtype, as this study attempted, appears to be a promising direction for clarifying inconsistencies in the existing literature on breastfeeding and breast cancer risk.

Conclusion

This survey-based study examined the relationship between breastfeeding duration and breast cancer occurrence among women previously diagnosed with the disease, with particular attention to how this relationship varies by molecular subtype. Longer breastfeeding duration was most consistently associated with HR+/HER2− breast cancer, a hormone-receptor-driven subtype, supporting the hypothesis that breastfeeding's protective effect operates primarily through reduced lifetime estrogen exposure. In contrast, HER2+ and triple-negative subtypes, which are not primarily hormone-driven, showed more variable breastfeeding patterns, suggesting that the protective association between breastfeeding and breast cancer risk may not be uniform across all disease subtypes. These findings suggest that breastfeeding duration, considered alongside molecular subtype, may offer a more nuanced modifiable factor for breast cancer risk reduction than previously appreciated. While the small, demographically limited sample restricts how broadly these conclusions can be applied, this study adds to the evidence supporting breastfeeding promotion as part of broader breast cancer prevention efforts and highlights molecular subtype as an important variable for future research in this area.

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The Use and Effectiveness of AI in Gut Microbiome-Autism Research: An Exploration
By Ema Tiefel

1. Abstract

Autism spectrum disorder (ASD), a neurological disorder that affects approximately 1 in every 31 children, has become more prevalent in recent years. While many factors have been confirmed to contribute to its development, it seems that, along with various other disorders, the gut microbiome plays a key role in the etiology of ASD more than previously expected. Children with ASD frequently suffer from metabolic and nutritional abnormalities and deficiencies, which are linked to their symptoms. Patients are found to have variations in gut microbiota compared to neurotypical controls, which may correlate with the presence of ASD. As the effort to better understand the complexities of ASD has yielded new advancements, AI offers new possibilities for visualizing and processing research data. However, its use in neuroscience remains limited.

This project aimed to utilize basic AI learning models to detect correlations and patterns between specific gut microbiota and autism, as well as represent the processed medical data in a visible format for interpretation. Three supervised learning models were created using a dataset containing gut microbiota samples from a cohort of 143 neurodivergent and neurotypical controls in order to organize strains by their decisiveness in determining whether or not the patient was neurotypical or had ASD. The insights provided by this project can provide an example of the ways in which AI may aid in better understanding the correlation between gut microbiota and autism spectrum disorder, and showcase its potential as a future research tool.

2. Introduction

ASD is a neurological disorder that affects an estimated 1 in 100 people globally and is present in 1 in every 31 individuals within the United States. Diagnoses have increased sharply over the past 20 years by up to 300%, thought to be driven primarily by public attention drawn to the disability, systemic reforms redefining medical terminology, and advances in neuroscientific research methodologies to better understand the nature of the disorder, though other outside forces yet to be considered may be contributing to its growing presence. According to the *Diagnostic and Statistical Manual, Fifth Edition (DSM-5)*, ASD is characterized by early manifestation in youth, persistent deficits in social communication, restricted and repetitive patterns of behavior, and symptoms causing clinically significant impairment in serious areas of the patient's current functioning (CDC, 2025).

ASD itself is a notoriously challenging disability to research, due to the fact that the sheer amount of variance in the manner ASD manifests from individual to individual can make the disability harder to diagnose promptly and treat based on a fixed and basic set of criteria. Some patients may exhibit clear behavioral and physical differences, while others, frequently dubbed as "high-functioning" individuals, may be harder to distinguish from their neurotypical peers. The etiology of the disorder also relies on a multitude of factors, many of which are poorly understood or difficult to measure and observe, such as pre- and perinatal circumstances, parental

health, exposure to certain compounds, and vitamin/elemental deficiencies. While genetics is considered to play a major role in the occurrence of autism, no singular gene can be pinpointed regarding its causation. However, recent research has revealed with certainty that gut microbiota play a definite role in the disorder, shedding light on the mechanisms behind ASD and potentially setting the trajectory of future research endeavours.

The gut microbiome is the collective sum of all microorganisms, including bacteria, fungi, and archaea colonizing the gastrointestinal (GI) tract at any given time. It has become a subject of growing curiosity in neurological and medical research as a result of its connection to human health, affect, and higher cognitive functions (Carabotti et al., 2015). It is part of a bidirectional communication network between the GI tract and the brain, partially responsible for regulating both the immune and nervous systems, and exerts a marked influence on homeostasis and disease (Thursby & Juge, 2017). An imbalance (dysbiosis) in the gut microbiome has been observed to strongly correspond with the pathogenesis of both physical and psychiatric issues, including anxiety, ASD, and depressive disorders, as well as physical problems such as diabetes, irritable bowel syndrome (IBS), and chronic inflammation. This system, spanning across the GI tract, the enteric, central, and autonomic nervous systems, the gut microbiome, the vagus nerve, the spinal cord, and the brain, is known as the gut-brain axis (GBA) (Carabotti et al., 2015). Theories of connections between the GI tract and the brain are not a novel concept, and have been present in medical research for a considerable time. In the mid-19th century, discussions about the gut microbiome already existed in England, Germany, and the United States. As early as 1998, oral introduction of a single, unique bacterium *(Campylobacter jejuni)* to rats in small doses was found to lead to anxiety-like behavior, without an accompanying immune response (Appleton, 2018; Lewandowska-Pietruszka et al., 2022). Furthermore, as recently as 2026, research has revealed that gut-brain communication may hold a key to reversing cognitive decline, identifying a critical link between the bacteria that live in the gut and the impairment often co-occurring with aging (Conger, 2026).

The idea that the GBA and gut microbiome influence mental and physical state has been repeatedly shown to extend to the etiology of neurodivergence and mental health. Apart from ASD, dysbiosis has been linked to various other neurological disorders such as generalized anxiety disorder (GAD), depression, Alzheimer's disease, eating disorders, and schizophrenia. Dysbiosis also causes physical health issues, such as Crohn's disease, ulcerative colitis, diabetes, and obesity, some of which experience significant overlap with ASD as comorbidities (Peralta-Marzal et al., 2021; Xiong et al., 2023). In fact, research indicates ASD patients consistently exhibit distinct differences in gut microbiota taxa compared to neurotypical individuals, which have subsequently been linked to neuroinflammation and compromised GI barriers ("leaky gut syndrome"). Approximately 40% of ASD patients suffer from GI symptoms such as diarrhea, constipation, vomiting, feeding problems, reflux, and abdominal pain. A comprehensive meta-analysis of multiple studies discovered that beyond harboring an altered gut microbiota, remodeling the gut microbiota in several cases reportedly alleviated ASD symptoms (Fattorusso et al., 2019; Grau-Del Valle et al., 2023; Mehra et al., 2022).

Despite the substantial role the gut-brain axis and gut microbiome play in ASD, little has been done to address the subject of microbiome-centered ASD research. Endeavours are at risk of being undermined by current limitations. The extreme genetic and medical heterogeneity of ASD makes reproducibility and consistency of previous results difficult, and has often led to different studies generating diverging outcomes. Studies often lack a uniform methodology, use varying cohort sizes, and may fail to account for influences on the gut microbiome that affect its composition, such as diet, geography, or medication. The complexity of the gut microbiome alone makes research harder, as advancing technologies have simultaneously allowed for higher resolution in the microbiome field but have also caused further divergence in results and created more variables to be accounted for (Saurman et al., 2020). The amount of research data yielded from analyzing microbiomes also poses an immense computational roadblock, as datasets include metagenomic sequences containing incredibly expansive quantities of DNA sequencing data. Metagenomics offers broader descriptions than older methods because it analyzes collective DNA sequences of all microorganisms present in a bulk sample (National Human Genome Research Institute, 2026). Computing and processing such quantities of data will prove to be incredibly difficult to perform through older means.

Lastly, the issue of data and information management for gut microbiome-ASD studies has posed another major hindrance to advancement. The plethora of data provided by metagenomic sequencing has led to problems with data organizations that frequently do not comply with Findable, Accessible, Interoperable, and Reusable (FAIR) standards for sharing and availability. An investigation of publications spanning human gut microbiome research and case studies of soil and gut microbiota found nearly half did not meet minimum standards for sequence data availability, and only 7% of authors provided usable data when contacted. Numerous studies also provided raw sequence data with insufficient or mismatched metadata, with additional information either disorganized or unavailable. Information is also fragmented across multiple documents and is difficult to publicly access due to a lack of centralized, unified databases; consequently, finding one comprehensive metagenomic dataset for ASD research is an incredibly challenging task (Kim et al., 2025).

As a result, finding a single tool to easily and quickly process and consolidate the large volumes of data generated in gut microbiota-ASD research remains difficult. To bridge the computational bottleneck, artificial intelligence (AI) and machine learning (ML) present a solution to the obstacles research currently faces. AI is inherently reliant on pattern recognition algorithms, deep learning, and predictive modeling, synergizing well with the demands of gut microbiota-ASD research in a time-efficient manner. The flexibility of AI allows it to be tailored to specific research uses by modifying and combining different types of learning models, which can target multiple analytical challenges simultaneously. ML models have already proven to be accurate in their predictions, versatile across multiple complex datasets, and able to serve multiple uses at once. Learning models have already been used to differentiate between ASD and neurotypical control groups by analyzing microbial differences and previously neglected smaller microbial communities with remarkable success and accuracy (Bašić-Čičak et al., 2024;

Olaguez-Gonzalez et al., 2023). This project aimed to test the efficacy of AI learning models and evaluate their capabilities in predicting and visually representing the correlation between different operational taxonomic units (OTUs) and the incidence of ASD in patients within a dataset.

3. Materials and Methods

An open-source dataset was obtained from Kaggle, a public platform and data repository. A cohort of 143 children aged 2-13 was selected for study, then evaluated using 16S rRNA sequencing to collect metagenomic sequencing data. Gut microbiota collected from ASD patients via fecal sampling was compared with a neurotypical control group (divided equally into 30 individuals per group). The dataset produced was utilised as training data for all three machine learning (ML) models in the research project. The gene changes in autistic subjects involved 1,312,364 analytes, contrasting with 1,334,835 analytes in neurotypical controls, showing that ASD subjects showed more unique variations in microbial genes. The amount of data provided on taxa was significantly increased in the ASD group compared to control groups when counting phyla and genera; however, ASD subjects had a decrease in species compared to a healthy baseline. ASD subjects had a higher degree of evolutionary groups within the microbiome yet seemingly had fewer individual strains present, a pattern uncharacteristic of neurotypical and/or healthy controls (Kotlik, 2020).

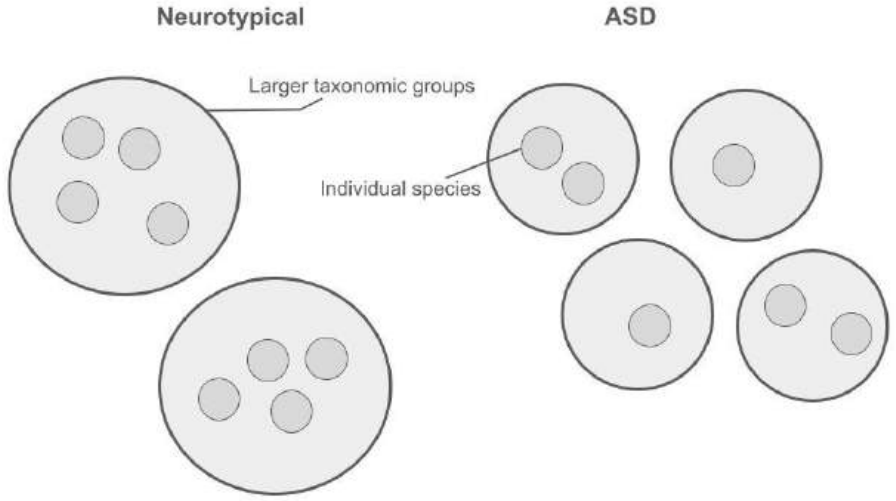


Fig. 1: Visual diagram of the difference in the number of taxonomic groups and individual species in ASD and neurotypical subjects

Three supervised learning models, a Random Forest Classifier (RFC), an XGBoost model (XGB), and a Logistic Regression model (LR), were then built to process and analyze the dataset. The dataframe was first cleaned of unreadable data that could potentially cause errors, then reoriented for machine learning purposes. The modified dataframe contained each OTU detected from the bacterial DNA sampled and sequenced in the study (indicated by the “OTU”

label on the Feature Importance graph), as well as the stool sample of each subject. The dataframe was divided into feature and target variables, then split between training (80%) and testing (20%) data. Data was stratified afterwards to ensure the number of neurotypical control and ASD samples remained proportional, and standard scaling was applied to adjust the numerical values within the dataset to equal proportions. Performances of all three models were evaluated following training by calculating their classification reports and displaying a confusion matrix, a precision-recall curve, and an (ROC) curve for each. Confusion matrices function by placing predictions into one of four categories: a true negative (TN) indicating properly classified negatives, a true positive (TP) indicating properly classified positives, a false positive (FP) indicating a negative misclassified as positive, or a false negative (FN) indicating a positive misclassified as a negative. A precision-recall curve graphically represents the trade-off between precision and recall within the model, while a receiver-operating characteristic (ROC) curve is a visual representation of model performance across all thresholds (Google, 2015).

The Random Forest Classifier (RFC) model was initially used as a baseline to compare other models against, as well as to test how a basic model would fare in accuracy. The model was fitted and trained using the processed and cleaned OTU data. The RFC model was selected as a baseline due to its basic structure compared to other learning models, requiring little tuning while creating relatively good prediction performance. RFCs are supervised ML algorithms that use an ensemble of unique decision trees created from random sections of data, which are then combined to create a stabler, more accurate prediction and reduce overfitting (*Random Forest Algorithm in Machine Learning*, 2026).

After a baseline of performance was established using the RFC model, an XGBoost model was created to test the capabilities of a more complex model. The same parameters for splitting, stratifying, and scaling the data were used for the XGBoost model, although the random state was adjusted to varying results. XGBoost models are, similarly to RFCs, ensemble learning algorithms. They use hierarchical tree structures to correct errors made by previous models and slowly strengthen themselves. An XGBoost model was selected due to the model's ability to handle larger amounts of data, as well as its speed, efficiency, and ability to scale well with large datasets. Boosting also creates robust models less prone to overfitting.

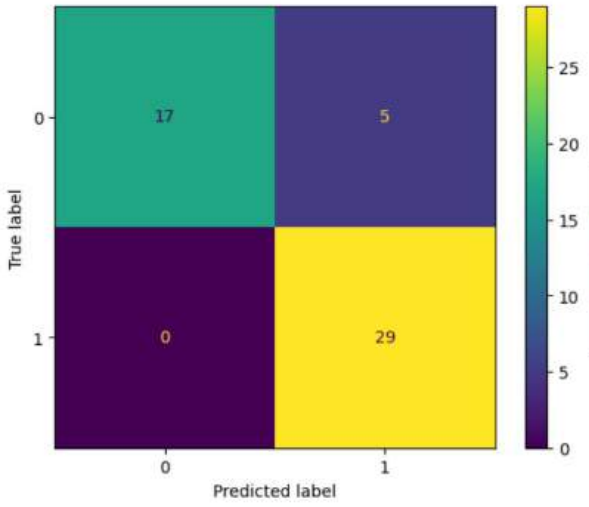
Lastly, a Logistic Regression model was implemented. LR was chosen for its common use, speed, and flexibility, despite its simplicity. Notably, unlike the previous two models, LR does not use decision trees for classification; it is a binary classification model reliant on mathematical equations to predict the probability that a sample belongs to one of two classes, which made it appropriate for determining whether or not a sample in the dataset was ASD or control. Therefore, LR was considered a favorable choice as a baseline classifier for slightly more advanced ML models. The LR model used the same parameters for splitting, stratifying, and scaling the data as the RFC model.

4. Results and Analysis

All three models operated as expected and finished training without error. Following the training of all three models, performance reviews were conducted using confusion matrices, ROC curves, and precision-recall curves. Three graphs were generated to visualize each model's classification outcomes, ability to discriminate between ASD and control samples, and balance between precision and recall. A more comprehensive analysis of the differences in each model's evaluation metrics is available in Fig. 11.

4.1 Random Forest Model Analysis

According to Fig. 2 below, the confusion matrix demonstrated that the Random Forest model performed relatively well in properly classifying the majority of ASD and control samples, with a relatively low number of false negatives or positives. Of the 51 samples classified, 17 control samples and 29 ASD samples were correctly predicted, while only 5 were misclassified. This indicated that the RF model performed well at classifying samples, with few errors, high accuracy, and relatively balanced predictions despite its simplicity. The results gathered from the classification report of the RF model were organized into a table next to the confusion matrix in Fig. 2. The classification report, as well as the classification reports of the two other models used in this project, excluded both support and the weighted average. Both were considered unnecessary to the overall report of the model's performance, and the macro average was used to represent both classes as equally as possible.



	Precision	Recall	F1 Score
0	1.00	0.77	0.87
1	0.85	1.00	0.92
Macro Avg	0.93	0.89	0.90

Fig. 2: Confusion matrix and classification report for Random Forest model

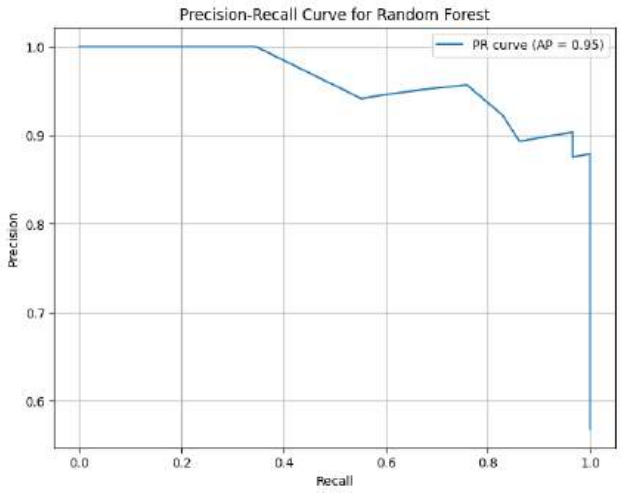


Fig. 3: Precision-recall curve for Random Forest model

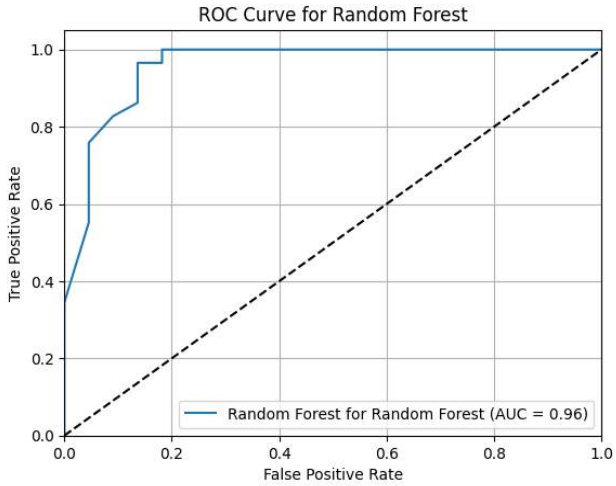


Fig. 4: ROC curve for Random Forest model

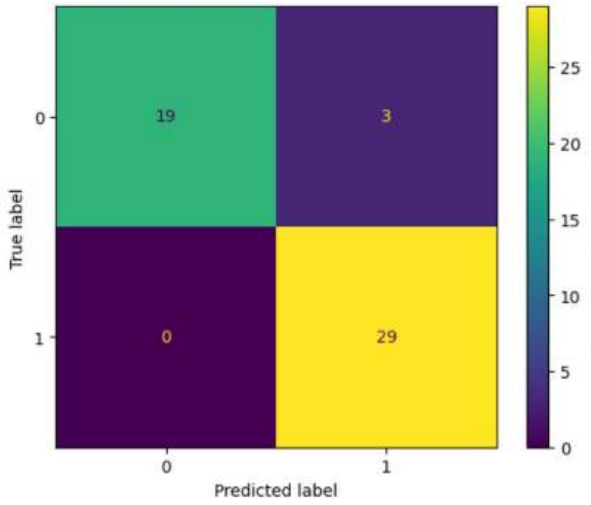
A difference was noted in the model's precision and recall for both categories. Heightened precision and low recall in control (0) samples were observed, yielding a 0.23 point difference, with a precision score of 1.00 and a recall score of 0.77. Meanwhile, lowered precision and heightened recall in ASD (1) samples were observed, yielding a 0.15 point difference, with a precision score of 0.85 and a recall score of 1.00. This indicated that the RF model likely predicted a control sample accurately more often, but remained conservative when predicting it; meanwhile, it was less apprehensive to predict an ASD sample, but was slightly less precise. The macro averages of both ASD and control samples were close to 1.00, supporting evidence that it functioned well across both classes equally. The high F-1 scores, an aggregate metric of both precision and recall, indicated the balance between both was stable. In Fig. 3, the precision-recall curve remained consistently high across a broad range of recall values and produced an Average Precision (AP) score of 0.95, showing that the model was able to

maintain relatively high precision while successfully identifying a large proportion of ASD samples.

Although precision appeared to decline when recall approached 1.0, the reduction was naturally expected as a result of the trade-off between overidentification and risk minimization, and did not negatively affect the performance of the model. The overall stability of the curve suggests that the model was able to maintain a desirable balance between minimizing false predictions and properly detecting ASD cases. The ROC curve (Fig. 4) produced a relatively high area under the curve (AUC) of 0.96. The shape of the graph and the high AUC value indicated that the model properly distinguished between ASD and control samples across a wide range of thresholds. Overall, the results of each evaluation established that the RF model met and exceeded expectations as a baseline model for comparison.

4.2 XGBoost Model Analysis

Compared with the RF baseline, the XGB model showed improvements across every evaluation metric. Fig. 5 displays the confusion matrix of the XGB model along with its classification report. Notably, improvements were observed in the accuracy of the model, including a reduction in misidentifications in both categories, producing 19 true negatives and 3 false positives. The classification report showed that model performance improved across both classes. While differences in precision and recall in ASD (1) and control (0) samples persisted, both classes experienced an increase in scores and a decrease in the gap between their precision and recall scores, respectively; additionally, the F-1 scores of both categories and the overall macro averages increased.



	Precision	Recall	F1 Score
0	1.00	0.86	0.93
1	0.91	1.00	0.95
Macro Avg	0.95	0.93	0.94

Fig. 5: Confusion matrix and classification report for XGBoost model

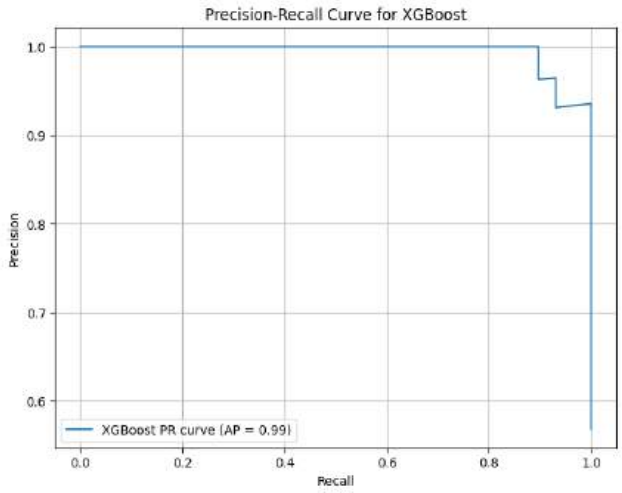


Fig. 6: Precision-recall curve for XGBoost model

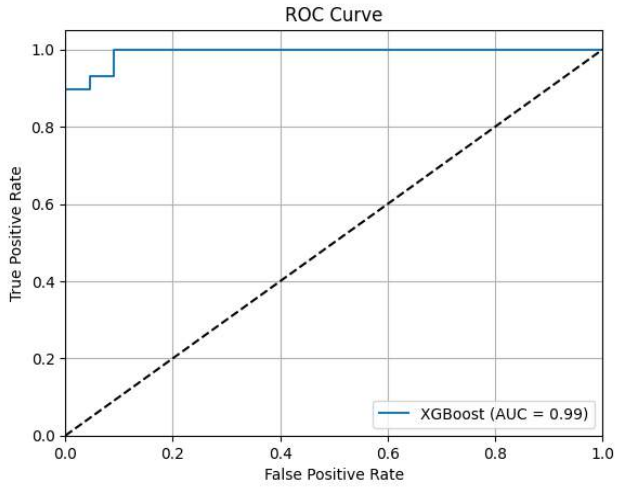
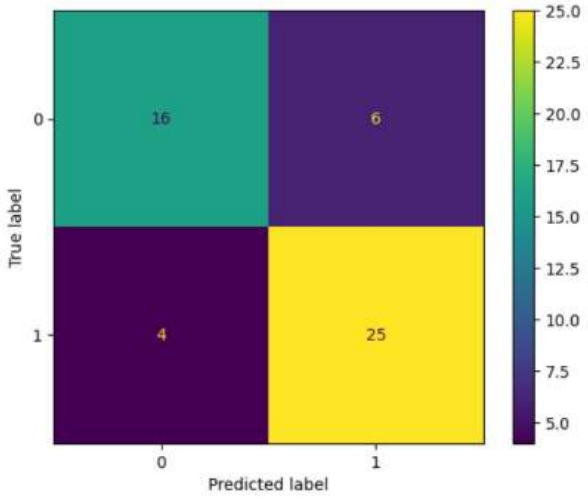


Fig. 7: ROC curve for XGBoost model

The PR curve (Fig. 6) of the XGB model was more stable and maintained consistently higher precision across a much broader range of recall values than the RF baseline, resulting in an AP score of 0.99, a 0.04-point increase. These results suggested that the XGB model was, on average, able to identify a greater proportion of ASD samples while minimizing the number of its false positive classifications. The ROC curve (Fig. 7)'s more stable shape demonstrated that the AUC totaled a value of 0.99, a 0.03-point increase from the original AUC of the RF model and only 0.01 point away from the maximum AUC value (1) a model can score, reflecting the XGB model's improved ability to predict across a wider range of thresholds without misidentification.

4.3 Logistic Regression Analysis

After comparison with both the RF baseline and the aforementioned XGB model, the Logistic Regression model was determined to be the least effective ML model out of all assessed. The confusion matrix and classification report (Fig. 8) show a clear increase in misidentification of samples and a decrease in correct classifications. Of the ML models used for this project, the LR model was the only one to have a false negative count above zero (4), and had the highest number of misidentified samples as well as the lowest number of accurately identified samples of all three. The macro averages, F-1 scores, and precision and recall scores of both ASD and control classes dropped noticeably, and no significant difference in the gap between either class's precision and recall was found. Furthermore, in its PR curve (Fig. 9), the LR model's precision remained stable under a marginally narrower range of recall values. The resulting curve was less stable and produced an AP score of 0.88; the decline was considered uncharacteristic of a natural precision-recall tradeoff. The results of the confusion matrix, classification report, and PR curve suggested the model struggled to minimize risk while identifying samples compared to both the baseline and the previously used XGB model.



	Precision	Recall	F1 Score
0	0.80	0.73	0.76
1	0.81	0.86	0.83
Macro Avg	0.80	0.79	0.80

Fig. 8: Confusion matrix and classification report for Logistic Regression model

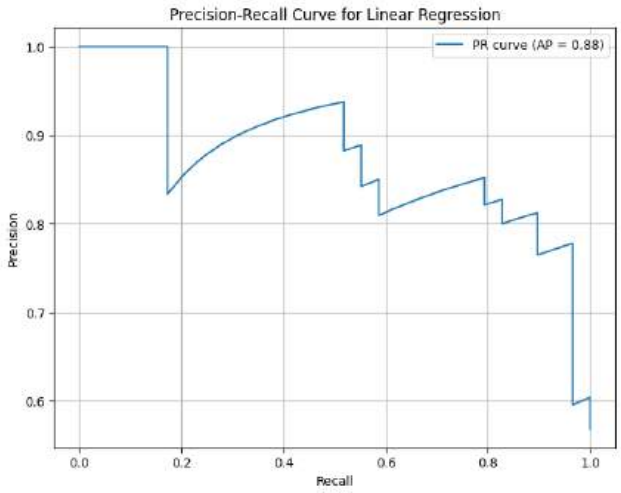


Fig. 9: Precision-recall curve for Logistic Regression model

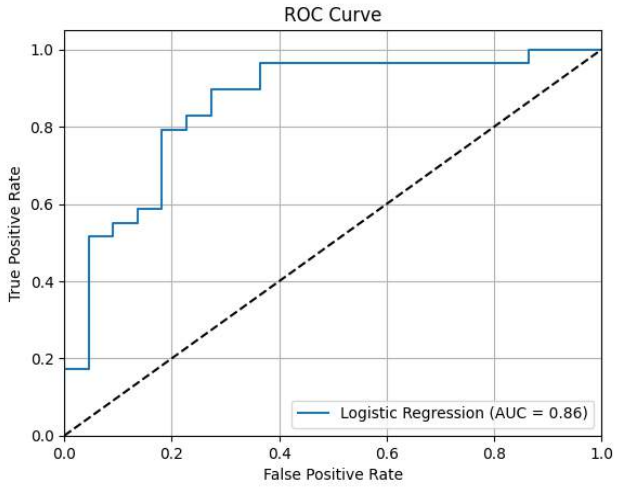


Fig. 10: ROC curve for Logistic Regression model

Accuracy at all thresholds (shown in Fig. 10) appeared to be in a relatively acceptable range for model performance, although the shape of the curve was less stable. The AUC was relatively high but small, with a value of 0.88. However, the model still performed below baseline and had the lowest AUC among the ML models used for the project. A general decrease was observed across all evaluation metrics used to measure the LR model's performance, indicating that it struggled to meet the baseline expectations and did not exceed or match those of the XGB model.

Metric	RF Baseline	XGBoost	Logistic Regression	Baseline Δ XGB	Baseline Δ Logistic Regression	XGB Δ Logistic Regression
Control Precision (0)	1.00	1.00	0.80	0.00	-0.20	-0.20
Control Recall (0)	0.77	0.86	0.73	+0.09	-0.04	-0.07
ASD Precision (1)	0.88	0.91	0.81	+0.03	-0.09	-0.10
ASD Recall (1)	1.00	1.00	0.86	0.00	-0.14	-0.14

Note: "Δ" indicates change between model scores.

Fig. 11: Reference table of value comparison between classes

4.4 Feature Importance and OTUs

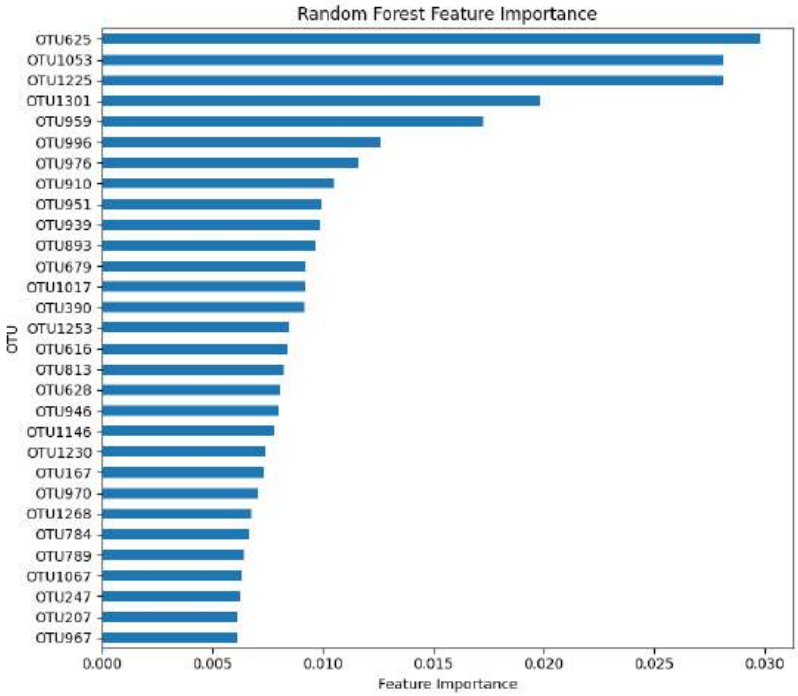


Fig. 12: Feature Importance graph for Random Forest model

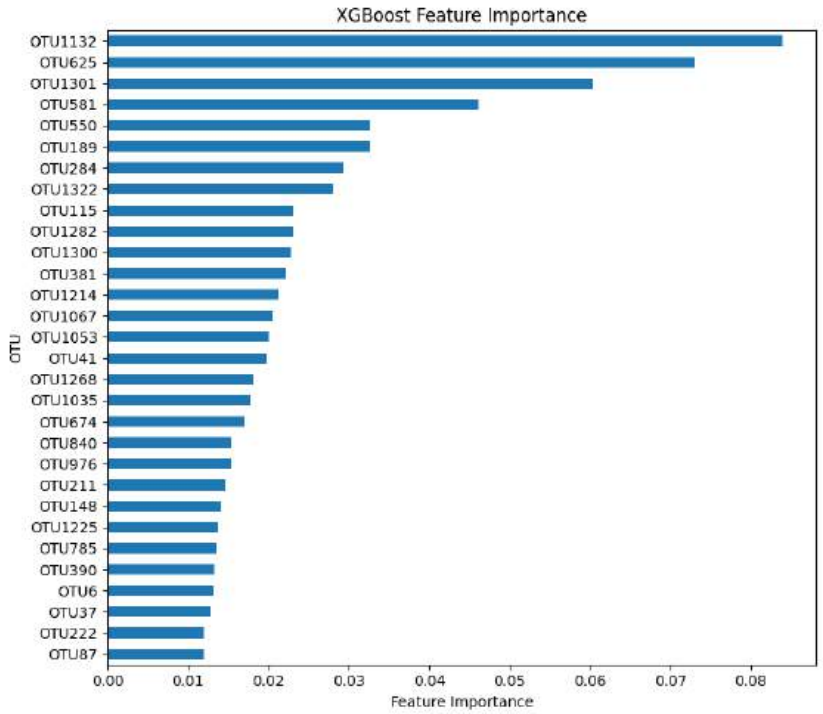


Fig. 13: Feature Importance graph for XGBoost model

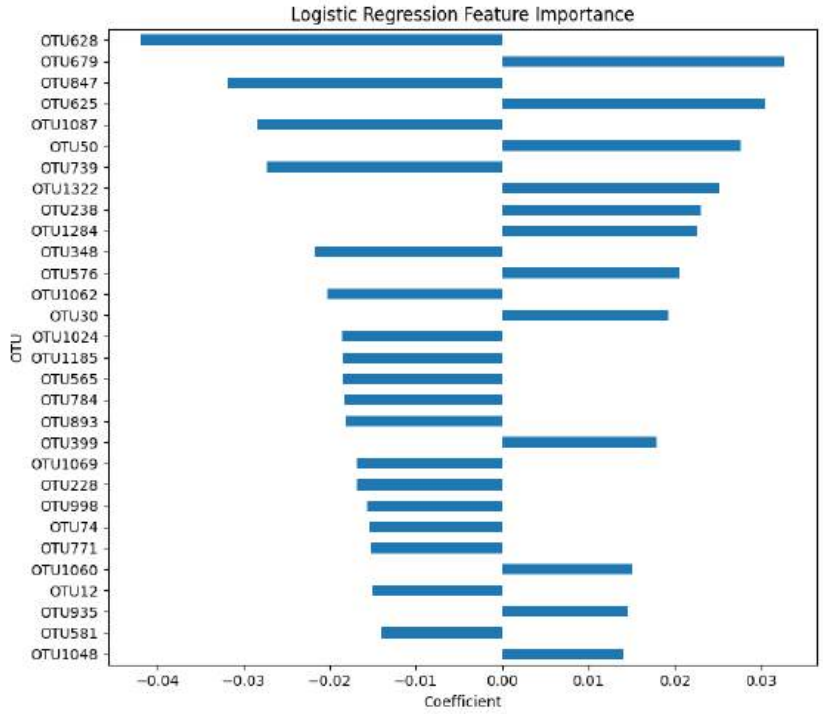


Fig. 14: Feature Importance graph for Random Forest model.

After all three evaluations were completed on the RF model, a series of Feature Importance (FI) graphs (Figs. 12-14) was created to discern which OTUs had the strongest influence on its predictions of whether a sample was ASD or control. For brevity and simplicity, the 30 most prominent OTUs were selected and displayed in order of decreasing contribution. Upon analysis, it was noted that OTU 625 was highly ranked in the feature-importance plots of the Random Forest and XGBoost (Figs. 12- 13), ranking first and second, respectively. The consistently high placement suggested it had been a particularly influential contributor to both models' predictions. A number of other OTUs were shared between the models, though placement was less consistent and broadly scattered (OTU 1053, 1225, 1301, 976, 390, 1258, 1067), and the plots had a collective overlap of approximately 27%.

The FI plot for the Logistic Regression model (Fig. 14) differed significantly from Fig. 12 and Fig. 13. The results revealed it prioritized vastly different taxonomic units than the prior models. Both baseline and LR shared five OTUs (628, 679, 625, 893, 784) and a 17% overlap compared to the XGBoost model, and their placements were also broadly scattered. This variance in OTUs suggests both models valued several integral parts but otherwise factored in completely different units when classifying samples. The LR model appeared to favor OTU 625 almost as highly as its baseline, ranking it as the fourth most important feature in its ability to predict sample class.

The cross-model consistency between the three frameworks was notable, as they appeared to highly prioritize the same feature despite relying on different forms of machine learning. The pattern suggested all models, regardless of their differences in function, found OTU 625 particularly informative in making a distinction between ASD and control samples. To investigate whether this discovery and model behavior would establish a pattern with pre-existing data in ASD-GBA research, OTU 625 was first 'counted' by calculating the average value of OTU 625 across both ASD and control samples. The ASD mean (~104.112) significantly outnumbered the control mean (~13.387) by nearly eight times. After calculating its average value, OTU 625 was examined to determine its taxonomy. 625 was classified as a member of the genus *Prevotella*. Literature was then examined concerning *Prevotella* and ASD.

A study in 2013 reported that autism and accompanying GI symptoms were characterized by lower levels of *Prevotella*, among several other bacteria (*Reduced Incidence of Prevotella and Other Fermenters in Intestinal Microflora of Autistic Children | PLOS One*, 2013). However, a separate, more recent 2021 study reported that a *Prevotella* sp. phylotype showed higher relative abundance in autism cases (Agarwala et al., 2021). The discrepancy in evidence surrounding *Prevotella* may stem from the fact that it is a genus, not a species, and therefore specimens of *Prevotella* may vary in abundance and impact. While it is unknown which specific species or phylotype OTU 625 may be, it is still important to note that its presence in all three models' FI plots, along with its higher mean value in ASD samples, warrants comparison to *Prevotella* linked with ASD and GI symptoms.

The reviewing process was repeated with the other shared OTUs, which were first categorized. Clusters were immediately noticeable, with the main phyla of the shared OTUs

being either *Bacteroidetes* or *Firmicutes*. Of the eleven remaining, eight belonged to *Firmicutes*. Within the phylum, a substantial concentration of the families *Ruminococcaceae* (628, 976, 1053) and *Lachnospiraceae* (**679, 784, 893, 1225**) was present, and only one OTU belonged to *Veillonellaceae* (1301). The remaining three OTUs belonging to *Bacteroidetes* had a different assortment of families, including *Porphyromonadaceae* (390), *Bacteroidaceae* (1258), and *Prevotellaceae* (1067), to which OTU 625 was also assigned. Afterwards, the abundance of each OTU across both ASD and control samples was calculated. Due to a lack of species-level assignments for the shared OTUs, each OTU was searched using its taxonomic family name.

Ruminococcaceae showed particularly interesting results, as multiple recent studies suggest the family to be associated with ASD. *Ruminococcaceae* is one of many butyrate-producing taxa affecting the production of key metabolites, thereby affecting brain function and behavior. ASD groups were repeatedly observed to have reduced abundance of these taxa, including *Ruminococcaceae,* pointing to a potential correlation between it and autism (Biagioli et al., 2025). The mean values of OTUs 628, 976, and 1053 were considerably lower in ASD samples; most notably, the control mean of 1053 was almost six times larger than the ASD mean of 1053. *Lachnospiraceae* is a large family of bacteria, and therefore appears to be both positively and negatively associated with ASD. Depletion of certain strains has been causally associated with ASD susceptibility and inflammation due to their association with immune defense (Zheng et al., 2026). However, overabundance of some unmarked *Lachnospiraceae* has been flagged as a potential risk factor (C. Chen et al., 2025; Zheng et al., 2026). Similarly, the results of the mean values of OTUs 679, 784, 893, and 1225 were largely inconsistent and did not seem to increase or decrease consistently in one class over another, and because of a lack of species-level classification, granular analysis could not be done. Regardless, the inconsistency still warrants comparison to *Lachnospiraceae*'s relationship to ASD.

Prevotellaceae is, similarly to *Lachnospiraceae*, both positively and negatively associated with autism. Studies have reported both increased and decreased abundance of *Prevotella/Prevotellaceae* in ASD (Z. Li et al., 2023; Sun et al., 2019). While the mean of OTU 625 increased sharply in ASD samples, consistent with literature suggesting an abundance of *Prevotellaceae* was associated with ASD, OTU 1067's mean nearly doubled in control samples instead. Similarly to the literature comparison done of shared OTUs in the *Lachnospiraceae* family, a species-level classification was not available, and therefore further granular analysis could not be done to confirm whether or not 625 and 1067's means matched research literature exactly specific to them.

The mean abundance of *Porphyromonadaceae, Bacteroidaceae*, and *Veillonellaceae* in both ASD and control samples was calculated and compared to literature regarding their relationship and association to ASD. Studies have pointed to elevated levels of *Porphyromonadaceae* inducing ASD-like behaviors in mice, and monitoring of monozygotic twins with ASD showed that the sibling with a higher abundance of *Porphyromonadaceae* suffered from more severe symptoms (Huang et al., 2026; B. Li et al., 2025). OTU 390, the only overlapping feature to belong to the family, did have a higher mean in ASD compared to control

by approximately four times. *Bacteroidaceae* is frequently studied in connection to autism, but also yields mixed results similarly to *Prevotellaceae* and *Lachnospiraceae*, as mentioned previously; while it has been observed more commonly in ASD groups by some studies, other studies suggest the differences in level may not be significant enough to warrant correlation (David et al., 2021; Ma et al., 2019). Similarly, OTU 1258's ASD mean did not substantially differ from its control mean. Lastly, reduced levels of *Veillonellaceae,* which is a family of bacteria associated with carbohydrate digestion and fermentation in the gut, have been linked to autism in multiple microbiome studies (Q. Chen et al., 2025; Fattorusso et al., 2019). OTU 1301's control abundance mean was nearly nine times that of its ASD abundance mean, matching the patterns established by previous ASD microbiome studies.

As only one OTU per family in *Porphyromonadaceae, Bacteroidaceae*, and *Veillonellaceae* was noted as significant enough to rank highly and overlap with the feature importance of all three ML models, a single OTU cannot completely determine if all instances belonging to the three families in the dataset accurately align with the literature reviewed. However, the commonalities between the literature and the results of the abundance mean of the three families still warrant some attention.

5. Discussion

To create the three ML models needed for this project, a publicly available Kaggle dataset was used which contained the metagenomic sequencing data taken from 60 subjects (30 control, 30 ASD) out of a larger cohort of 143 subjects aged 2-13. The data was prepared and processed by the baseline Random Forest, XGBoost, and Logistic Regression models, after which the results were used to showcase each model's ability to properly classify a sample as ASD or neurotypical. The tree-based models, RF and XGB, were the most successful, while the equation-based LR model appeared to perform below baseline. After reviewing the performance, a feature importance graph was made for each model to show which OTUs contributed the most to each model's decisions, and any overlap between them was noted. Upon examining the highest-ranking OTUs and classifying them by viewing their taxonomy information within the dataset, the overlapping OTUs were compared to pre-existing literature regarding the families of each OTU in association with ASD. Nearly all of the OTUs matched prior studies, showing that information could be gleaned not only from the classification abilities of the models, but from the data itself.

It is best to note that while specific OTUs may have played a significant role in the model's prediction of a sample as ASD or control, the correlation between the OTU and an ASD diagnosis by the model does not equate to causation. Compared to the full extent of the gut microbiome, the numbers of OTUs examined were also not representative of its entirety. This does not mean the information provided by the models should be discounted, however; the model's predictions as well as the insights their workings provided were practical and lined up with pre-existing findings regarding ASD-GBA research. The performance of three ML models alone is not a completely accurate representation of each type's ability to process ASD-GBA

research data. However, the models were able to successfully showcase the potential and capability of machine learning in ASD.

6. Conclusion

ASD diagnoses have grown in recent decades, and as the disability has become more visible, research surrounding the disability and its extremely complex etiology has seen a renewed vigor. As discoveries are made regarding the origin of ASD and its development, new pathways to treat, diagnose, and analyze have been opened, one of which has been the treatment of ASD through the gut-brain axis and the gut microbiome. Despite the promise ASD-gut microbiome research holds, the amount of data that comes from such research may hamper future progress in this field. Data mishandled by human researchers may, through no fault of their own, become heavily disorganized and lost. This has led to a decentralization of information and a lack of accessibility. However, AI may be a solution to this computational issue. Machine learning algorithms are reliant on pattern recognition and data organization by design, and can be augmented to fit multiple research needs at once.

This project aimed to first showcase the versatility of AI in the context of ASD-GBA research by using three different machine learning models. The performance of all three models was tested and compared to one another to provide an example of AI's flexibility and the different ways in which it could be adjusted to fit specific research needs, as well as the accuracy of each model's prediction abilities. Afterwards, to show that the predictions of the model properly tied into pre-existing data regarding ASD and gut microbiota, the features most contributory to each model's decisions to classify its samples as ASD or neurotypical (control) were identified by name, then compared (and matched) to existing literature regarding their taxonomy and relationship to ASD.

ASD research has been important and will continue to be in the future. Recognizing machine learning as a tool to help address persisting issues of data management and organization will help prepare ASD-GBA research for a technologically complex age of medicine. Its usefulness lies in its ability to efficiently organize data and quickly find patterns within it, even on a small scale. While AI is not a perfect solution on its own, it is important to fully recognize its potential as a tool to clear the complexities of an area of study which will likely grow as time goes on. Future research endeavours involving the use and review of AI and machine learning in ASD-GBA and gut microbiome research may offer further insight into not only ASD and the gut, but how to streamline the research process itself.

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Machine Learning for EEG-Based Diagnosis of Depression and Anxiety: High Reported Accuracy but Uncertain Reliability By Srikar Kesanam

Abstract

Psychiatric diagnosis still rests largely on clinical interviews, an approach that depends heavily on patient self-report and clinician judgment and produces inconsistent results even for common conditions. Electroencephalography (EEG) paired with machine learning has been proposed as an objective alternative diagnostic approach, and individual studies routinely report classification accuracies above 95 percent for depression and anxiety. This review examines the reliability of diagnostic accuracies, organizing the evidence around which EEG features best separate affected individuals from healthy controls, which models perform most reproducibly, and which methodological factors decide whether a reported accuracy means anything outside the study that produced it. Signal complexity measures and classifiers such as the support vector machine and deep learning architectures perform well, but the headline figures come disproportionately from small, single-site samples and validation schemes of uneven rigor. When models are tested on patients they have never seen, performance tends to fall. EEG-based classification is promising, but it has not yet been tested the way a diagnostic tool needs to be tested.

INTRODUCTION

Diagnosing psychiatric illness largely rests on conversation and clinical interviews. Mental healthcare clinicians interview patients, ask about their mood, sleep, and appetite, and determine diagnoses by comparing responses against a checklist of symptoms in a manual such as the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), the standard reference used to diagnose mental illness (American Psychiatric Association). This approach is effective but relies heavily on two things that are hard to control: the patient's ability to describe their own inner state accurately, and the individual judgment of the clinician doing the assessment. Two physicians can see the same patient and reach different conclusions, and a patient having a good day can mask a serious problem. This is not a hypothetical concern. In the DSM-5 field trials, the diagnosis of major depressive disorder reached only low to questionable test-retest reliability, with a kappa of 0.28, meaning two clinicians assessing the same patient agreed only modestly more often than chance would predict (Regier et al.). For a field responsible for conditions as common and as serious as depression, which affects more than 300 million people worldwide (de Aguiar Neto and Rosa), that degree of subjectivity is a significant limitation.

Electroencephalography (EEG) is a technique that records the brain's electrical activity through sensors placed on the scalp, and it offers an appealing alternative to the clinical interview because it eliminates subjective interpretation entirely. EEG is non-invasive, comparatively inexpensive relative to other neuroimaging methods such as functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG), and it records brain activity

directly, with no self-report step in between (Elnaggar et al.). Crucially for a computational approach, EEG produces quantitative signals that can be fed straight into a model. The general pipeline is consistent across much of the literature. Raw EEG data are recorded and cleaned of noise, specific features are extracted from the signal, and those features are passed to a machine learning or deep learning classifier that outputs a diagnostic decision (Figure 1).



Figure 1. The EEG-based diagnostic pipeline.

The results of this computational diagnostic approach have been striking. They are also hard to trust. Individual studies routinely report diagnostic accuracies above 95 percent, suggesting a strong solution to the existing problem (Elnaggar et al.). However, those accuracy rates were determined from assessments using small sample sizes, inconsistent methods, and validation schemes that vary in rigor, which raises the question of how much of the reported accuracy comes from the brain and how much comes from the way the studies were built.

To better understand this computational diagnostic approach and its efficacy, this review examines how reliably machine learning and deep learning models can classify depression and anxiety from quantitative EEG features. Three specific questions were developed. The first asks which EEG features and biomarkers most consistently separate affected individuals from healthy controls. The second asks which models achieve the strongest and most reproducible performance. The third asks which methodological factors determine whether reported accuracy holds up as a real diagnostic signal. Coverage across the two disorders is uneven. Depression has been studied far more than anxiety, so the conclusions below are firmer for depression.

METHODS

Sources for this review were identified through Google Scholar and PubMed, chosen because together they cover the biomedical and engineering literature where EEG-based classification work is published. Searches combined terms describing the signal, the computational approach, and the disorders of interest, using strings such as “EEG” with “machine learning” or “deep learning,” paired with “depression,” “anxiety,” “classification,” or “biomarkers.” Boolean combinations were used to narrow results, for example “EEG AND deep learning AND depression” and “EEG AND anxiety AND classification,” and the reference lists of the most relevant papers were scanned for additional studies the keyword searches missed.

Inclusion and exclusion criteria were set before screening began. To be included, a study had to use EEG as its primary signal, apply machine learning or deep learning methods to the classification of depression or anxiety, be written in English, and be published between 2014 and 2025. Both original classification studies and review or systematic mapping papers were eligible,

since reviews provide the cross-study synthesis that individual papers cannot. Studies were excluded if they relied on a different modality such as fMRI or facial analysis, if they addressed EEG or the disorders without any computational classification component, or if they fell outside the language and date limits.

Selection proceeded in stages. Titles were screened first to remove clearly unrelated results, such as studies of unrelated neurological conditions or EEG work with no diagnostic aim. Abstracts of the remaining papers were then read to confirm that each one used EEG features with a machine learning or deep learning classifier and addressed at least one of the target disorders. Papers that passed abstract screening were read in full, and a final set of six core sources was retained based on how directly they spoke to the three research questions and how well they represented the range of features, models, and methodological approaches in the field.

Data was extracted from each retained source in a consistent format. For every study, the following were recorded: the EEG features or biomarkers used, the type of classifier applied, the sample size and composition, the reported accuracy or other performance metrics, the validation method, and any limitations the authors named. The extracted information was then organized by feature type, by model type, and by methodological quality, which is how the three sections of the analysis are structured. Because coverage of the two disorders in the retained sources is uneven, with depression better represented than anxiety, this imbalance is noted throughout so the reader can judge where the evidence is strong and where it is thin.

RESULTS

Across the retained literature, one broad finding recurs: EEG-based models can separate individuals with depression or anxiety from healthy controls, and they frequently do so at high reported accuracy. For depression, accuracy values in the range of 95 to 99 percent are common (Elnaggar et al.). Anxiety is represented by fewer studies, but the ones that exist report comparable performance, with one purpose-built model reaching 99.48 percent accuracy for generalized anxiety disorder (Liu et al.).

The evidence is not spread evenly, and the distribution itself is a finding worth recording. Depression dominates the literature by a wide margin, both in the number of studies and in the range of features and models tested against it. Anxiety appears far less often, and where it does, the studies tend to be one-off proofs of concept that no one has replicated. Reported accuracy also spans a wide band once methodology is taken into account, from the mid-70s to a perfect 100 percent, and sample size and validation method account for much of that spread.

The sections below take the features first (Figure 2), then the models and their reported accuracies (Table 1), then the study designs that produced those accuracies (Figure 3).

DISCUSSION

EEG Features and Biomarkers

Before any model can classify a disorder, researchers first need an EEG feature that tracks it. The literature converges on four broad families of candidate biomarkers, band power, alpha asymmetry, signal complexity, and network structure, though disagreement persists. Figure 2 compares the four families of biomarkers and replication consistency.

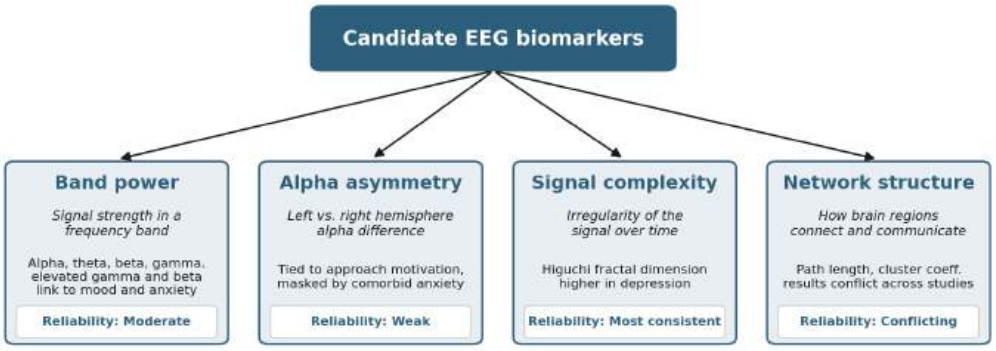


Figure 2. Four families of candidate EEG biomarkers for depression and anxiety. Sources: de Aguiar Neto and Rosa; Fitzgerald and Watson; Liu et al.

First, band power, or the strength of the EEG signal within a given frequency range, is the most direct and most studied feature for disorder classification. Two bands recur throughout the depression literature - alpha and theta. Alpha activity (roughly 8 to 13 Hz) is associated with a relaxed, idling brain, while theta activity (roughly 4 to 8 Hz) is linked to drowsiness and emotional processing. Hosseinifard and colleagues found that by analyzing alpha and theta power, especially in the left hemisphere, depressed patients could be reliably separated from healthy controls. In that same work, band power features alone performed less well than nonlinear complexity features, and combining the two raised classification accuracy to 90 percent (Hosseinifard et al.). A separate review of gamma band activity (roughly 30 to 50 Hz), a fast rhythm tied to attention and sensory processing, found consistently elevated gamma power in depressed patients and proposed it as a marker of mood instability (Fitzgerald and Watson). Beta power (roughly 13 to 30 Hz), a rhythm associated with active, alert thinking, carries similar weight for anxiety. Liu and colleagues found that classification accuracy for generalized anxiety disorder rose steadily as the EEG band shifted higher, with beta power alone reaching 97.45 percent classification accuracy. Liu posits that this reflects beta's role in attention, cognitive control, and emotion regulation, the same processes disrupted by anxious mood swings (Liu et al.).

Second, alpha asymmetry, which is the relative difference in alpha power between the brain's left and right hemispheres, has drawn particular attention as a marker of depression. This is because increased left-hemisphere alpha reflects reduced left-frontal activity and a corresponding lack of approach motivation (de Aguiar Neto and Rosa). However, a meta-analysis of this literature concluded that alpha asymmetry has little diagnostic value and is better suited to predicting treatment response than to diagnosis itself. This is due in part to the fact that studies vary so much in sample size that many never reach statistical stability (van der

Vinne et al.). The anxiety literature complicates the picture further, as one study found that anxious apprehension actively masks the relationship between depression and prefrontal alpha asymmetry. The same biomarker therefore behaves differently depending on the patient's condition and any combination of disorders present (Nusslock et al.). Therefore, alpha asymmetry is a second potential biomarker for diagnosing anxiety and depression but additional studies are needed to better understand its utility in diagnosing mental health conditions.

Third, signal complexity features, particularly Higuchi fractal dimension (a measure of how irregular or jagged an EEG signal looks over time), produce more consistent results across studies. Prior work reliably finds that depressed patients tend to have higher fractal dimension values than healthy controls. This finding is replicated across multiple studies and treated as one of the more dependable non-linear biomarkers in the literature (de Aguiar Neto and Rosa).

Finally, network features, which describe how efficiently different brain regions connect and communicate with one another, are messier than the findings for band power, alpha asymmetry, and signal complexity. Several studies agree that depressed brains show a more random, less organized network structure overall, but the specific graph measures used to demonstrate this point in different directions. For example, one study of 87 depressed and 58 healthy subjects found longer path length and lower cluster coefficient, two measures of how efficiently brain regions connect to one another, in depression. A separate study of only 13 depressed and 13 healthy subjects found the reverse. One sample is ten times the size of the other, which de Aguiar Neto and Rosa treat as part of the explanation. Additional conflicts across the literature are largely attributed to inconsistent electrode setups, differing band definitions, and small, unevenly matched samples, which make direct comparison between studies unreliable even when the studies agree on which feature to measure (de Aguiar Neto and Rosa).

Models and Classification Performance

Once a set of EEG features has been chosen, the feature set can be run through very different kinds of classifiers. The literature splits into two camps: classical machine learning models trained on hand-engineered features, and deep learning models that learn their own features directly from the EEG. Table 1 reports the representative studies, together with their samples and reported accuracies.

Support vector machines (SVMs) are classifiers that find the boundary separating two groups of data points. SVMs are the most heavily used classical algorithm for EEG-based depression detection and one of the strongest performers (Elnaggar et al.). Mumtaz and colleagues trained SVM, logistic regression, and naive Bayes classifiers on synchronization likelihood, a measure of how similarly activity in different EEG channels rises and falls together, and found that SVM reached 98.0 percent accuracy separating depressed patients from healthy controls based on EEG data alone (Mumtaz et al.). Other classical models, including k-nearest neighbors, logistic regression, and ensemble methods like random forest, appear throughout the same literature and perform well, though rarely as consistently as SVM. In one review, ensemble and neighbor-based methods ranged from roughly 79 to 97% accuracy depending on the specific

classifier and dataset (Elnaggar et al.). SVM does not swing that widely. Likewise, Wang and colleagues identified the same pattern in an unrelated EEG emotion-classification study, where linear SVM outperformed other kernel types and other feature types entirely (Wang et al.). Together, these results suggest that SVM's strength reproduces across datasets and across disorders.

Table 1. Representative EEG-based classification models for depression and anxiety.

Study	Disorder	Classifier	Sample (patients/ controls)	Reported accuracy
Mumtaz et al.	Depression	SVM	34 / 30	98.0%
Hosseinifard et al.	Depression	SVM + nonlinear features	45 / 45	90%
Seal et al. (DeprNet)	Depression	CNN	18 / 15	99.3% / 91.4% *
Zhang et al.	Depression	Graph CNN	two datasets	92.8% / 83.1% **
Liu et al. (MSTCNN)	Anxiety (GAD)	Multi-scale CNN + attention	45 / 36	99.48%
Wang et al.	Emotion (reference)	Linear SVM	6 subjects	87.5%

* Record-wise vs. subject-wise split.
** Dataset 1 vs. dataset 2.
Accuracies come from different datasets and are not directly comparable.

Deep learning models arrive at the same conclusion by a different route. SVM depends on features a researcher designs by hand; these models learn their own features straight from the signal. The two architectures that dominate this side of the literature are both types of deep learning. Convolutional neural networks (CNNs) scan small patches of an input for local patterns before combining them into a fuller picture, and long short-term memory networks (LSTMs) are built to retain information across longer stretches of a sequence. CNNs, LSTMs, and hybrids of the two dominate the mental health from EEG literature, with reported depression classification accuracies regularly above 98% (Elnaggar et al.). For anxiety, the evidence base is sparse, but one purpose-built model has strong support. The multi-scale spatial-temporal convolutional neural network (MSTCNN) of Liu and colleagues, which extracts spatial and temporal EEG features in parallel and adds an attention mechanism to weight the most informative channels and time points, reached 99.48% accuracy when classifying 45 generalized anxiety disorder patients against 36 healthy controls. The MSTCNN model outperformed EEGNet, a multi-resolution CNN, and a standard CNN-LSTM baseline tested on the same data (Liu et al.). Both the classical and deep learning approaches can distinguish patients from controls at high accuracy under study conditions, and the choice between them depends largely on how much data is available and whether a researcher wants to design features by hand or let the model find them.

Reviews of EEG-based depression and anxiety classification generally agree that SVM remains competitive with well-engineered features, which require considerable domain expertise and manual effort to design, but that deep learning approaches gain an edge when they have access to enough raw data to learn their own representations (Elnaggar et al.). Where these accuracy figures cannot converge, however, is whether a given model would still work on a different sample of patients.

Methodological Validity and Reliability

The accuracy figures reviewed above are only as meaningful as the study designs that produced them, and those designs share a set of weaknesses that inflate performance in predictable ways. Sample size is the most obvious. Many EEG depression studies work with fewer than twenty subjects in total, and only a handful exceed two hundred (de Aguiar Neto and Rosa). Beyond statistical power, with a small sample, a model can reach near-perfect accuracy by learning quirks specific to the individuals in the dataset rather than anything about the disorder. The alpha asymmetry literature makes the cost concrete. One meta-analysis estimated that roughly 300 subjects are needed before frontal alpha asymmetry produces a stable, biologically plausible effect in depression, which means a substantial share of published asymmetry findings were underpowered from the start (van der Vinne et al.). Liu and colleagues name the same problem in their own anxiety work, noting that 45 patients and 36 controls limit how confidently their 99.48% accuracy can be said to generalize (Liu et al.).

How a model is validated matters just as much as how many people it was trained on. The distinction that does the most work here is between splitting data by individual recording and splitting it by subject. In a record-wise split, segments from a single patient can end up in both the training set and the test set, which lets the model recognize the person instead of the condition. This is data leakage. In a subject-wise split, all of a patient's data stays on one side of the divide, so the model is only ever tested on people it has never seen. Seal and colleagues' DeprNet, a convolutional neural network built to detect depression from EEG, makes the gap obvious. Split by record, the model reached 99.3% accuracy. Split by subject, which is the clinically meaningful test because a real diagnostic tool encounters patients it has never encountered, the same model dropped to 91.4% (Seal et al.). Nearly eight points of apparent performance came from the split alone. The same concern applies across models. Zhang and colleagues' graph convolutional model reached 92.8% accuracy on one public dataset and fell to 83.1% on a second (Zhang et al.). A number measured on one sample says very little about the next. Figure 3 places the two drops side by side.

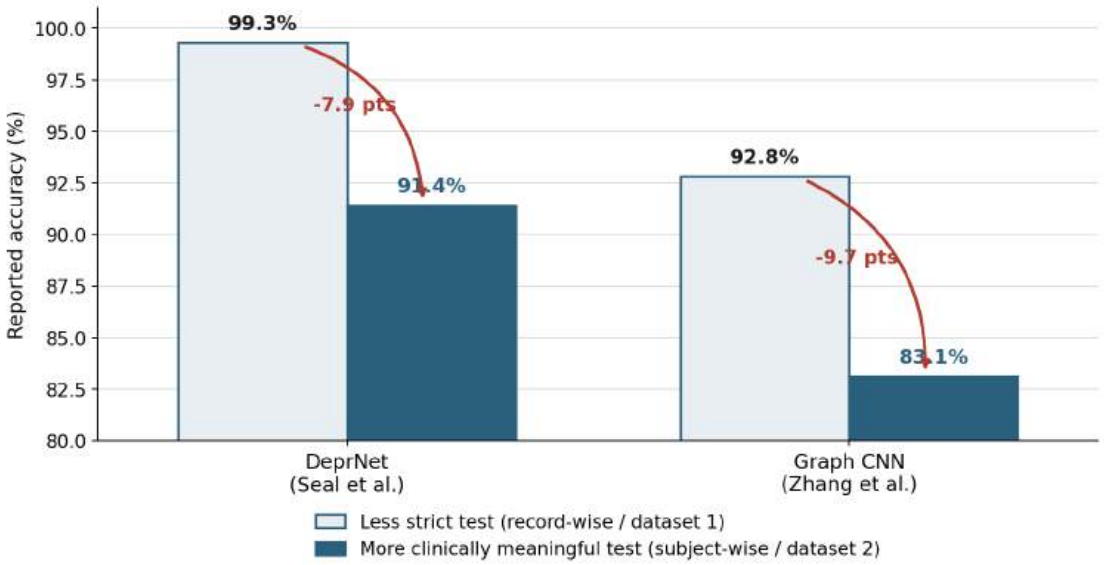


Figure 3. The same models score lower under stricter validation.

What rigorous validation looks like can be seen in work outside the mood-disorder literature. SCORE-AI, a clinical EEG model for interpreting routine recordings, fixed its architecture and decision thresholds before validation began and allowed no adjustments afterward, then tested the frozen model on recordings from centers that had taken no part in its development (Tveit et al.). Trained on more than 30,000 recordings and externally validated on nearly 10,000 more, it reached an area under the curve between 0.89 and 0.96 across categories of abnormality, placing it within the range of agreement seen among human experts (Tveit et al.). A frozen model tested on outside data is what separates a diagnostic signal from an artifact of study design, and it is almost entirely absent from the depression and anxiety literature reviewed here, where models are typically developed and evaluated on the same small, single-site dataset.

The field is aware of this. Recent reviews identify the shortage of large, diverse, standardized EEG datasets as a central research gap and note that most models perform poorly when moved across populations (Elnaggar et al.). This matters because a diagnostic tool that only works on the dataset it was built from is of little use in a clinic that sees different patients on different equipment. Whether the high accuracies reported for depression and anxiety reflect real neural differences or the narrowness of the data they were measured on remains, for now, an open question.

LIMITATIONS

Several limitations bound the results of this review. First, there is a lack of standardized datasets and uniform preprocessing methods across studies. Because different research groups record EEG with different equipment, numbers of electrodes, reference points, and definitions of where one frequency band ends and the next begins, there is substantial heterogeneity across the literature, which makes it difficult to draw direct comparisons across papers (de Aguiar Neto and

Rosa). Two studies can report the same feature and still not be measuring quite the same thing, which makes their accuracy numbers hard to compare.

Second, when methods vary this substantially across papers, pooling results into a single clean verdict becomes difficult. The field has no shared benchmark dataset that would allow competing models to be tested head to head under identical conditions. Recent work names this absence of large, diverse, standardized datasets as one of the central obstacles to progress in using EEG for mental health diagnostics (Elnaggar et al.). The high accuracies scattered through the literature are therefore hard to rank against one another with confidence, because the differences between them may reflect the data as much as the models.

Third, there is a limitation inherent to any review, which synthesizes existing work and collects no new data. A review inherits every weakness of its sources without the ability to correct them. If the studies reviewed here share a common blind spot, such as small samples or loose validation, this review can highlight the limitation but cannot fix it, and its conclusions are bounded by the quality of the evidence available.

Finally, the literature assessing EEG for the diagnosis of mental health conditions is modest in size and weighted heavily toward depression. Anxiety is represented by comparatively few studies, which means the conclusions drawn about it rest on a narrower and less replicated foundation than those drawn about depression. Broader coverage of anxiety, and of the methodological range within it, would give the comparisons in this review more weight.

FUTURE DIRECTIONS

Future work should aim to address the limitations of the field, beginning with the need for larger, standardized, multi-site datasets. Models trained and tested on a few dozen patients from a single center cannot be expected to generalize to the full range of people with mental health conditions, and the current shortage of large shared datasets is repeatedly named as a bottleneck (Elnaggar et al.). Data collected across multiple sites, with consistent equipment and preprocessing, would help researchers separate the neural signature of a disorder from the quirks of one lab's setup.

Validation practices need to tighten alongside the expanded data. As discussed above and shown in Figure 3, a model's reported accuracy can swing by several percentage points depending only on whether it was tested on unseen patients or on recordings it had partly seen before. Consistent use of leave-one-subject-out cross-validation, in which the model is always evaluated on people held out entirely from training, along with clear reporting of any leakage risk, would make published numbers much easier to compare. The value of fixing a model before validation and testing it on genuinely external data, as demonstrated in clinical EEG work outside the mood-disorder literature (Tveit et al.), offers a clear template worth adopting.

Beyond data and validation, there is room to move the research closer to real conditions. Most studies collect EEG in controlled resting or task settings, but a diagnostic tool would ultimately have to work in the messier context of everyday clinical practice. Extending this work toward more naturalistic recording settings, such as a home or a school, and toward continuous

or portable monitoring rather than one-off lab sessions, would help connect the promising accuracy figures to a tool a clinician could actually use.

CONCLUSION

The individual findings reviewed here are encouraging. The body of evidence behind them is not yet strong enough to call EEG-based machine learning a dependable diagnostic tool. Certain features replicate across studies, particularly signal complexity measures like Higuchi fractal dimension, classifiers such as SVM and modern deep learning architectures reach high accuracy, and at least one purpose-built model shows the approach can work for anxiety as well as depression.

Answering the three research questions means weighing the results of the included studies against how those results were produced. Reported accuracies above 95 percent are real, but they come disproportionately from small samples, single-site data, and validation schemes that sometimes let a model see its test subjects in advance. When the testing is done properly, on patients the model has never encountered, the numbers tend to fall, and the field still lacks the large standardized datasets that would settle how much of the reported performance reflects a true diagnostic signal.

EEG combined with machine learning can therefore distinguish affected patients from healthy controls under study conditions, but whether it can do so reliably in the clinic remains unproven. Closing that gap is mostly a matter of changing research practice. Larger multi-site datasets, validation that reports its own leakage risk, and testing that mirrors real clinical use would turn strong laboratory numbers into evidence a clinician could act on. Until then, EEG-based classification is best understood as a serious and fast-moving line of research that has not yet earned a place in routine diagnosis.

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Reducing Bio-AgNP Ecotoxicity with Post-Biosynthesis Physicochemical Modifications

By Danyan Yang

Abstract

This study analyzed effects of post-biosynthesis physicochemical modifications on ecotoxicity levels of biogenic silver nanoparticles (bio-AgNPs). Specifically, thiol-based ligand exchange with cysteine was tested to reduce bio-AgNP ecotoxicity, mitigating environmental detriments. Although biosynthesis appears to be a sustainable solution against conventional AgNP pollution, research is direly lacking in the field of post-biosynthesis physicochemical modifications mitigating bio-AgNP ecotoxicity. Thus, this experiment analyzed Escherichia coli growth upon exposure to modified and unmodified bio-AgNPs, synthesized from Fusarium oxysporum. Modified bio-AgNPs showed no significant difference in ecotoxicity compared to their unmodified counterparts, causing failure to reject the null hypothesis. The alternative hypothesis—that modifications mitigate ecotoxicity—lacked support. However, limitations included equipment challenges and the inherently narrow scope of interactions studied. Nonetheless, these findings help researchers understand how surface-level modifications are insufficient for enhancing AgNP safety and sustainability, highlighting the need for fundamental changes to AgNP production to protect both human and environmental health.

Introduction

Silver nanoparticles (AgNPs) are based on elemental silver and possess nanoscale structural dimensions. Unique physicochemical properties give these particles pivotal roles in medicine, electronics, consumer goods, and numerous other industries today (Beyene et al. 2017). However, AgNPs simultaneously exacerbate environmental degradation in countless ecosystems, which cannot adapt to lethal ecotoxicity entailed by exposure to silver ions that are persistent, bioaccumulative, and extremely toxic (Yan and Chen 2019; Ihtisham et al. 2021). Consequently, dire concerns arise around safety and sustainability implications of AgNPs for humans and the environment.

Increasingly dire concerns around ecotoxicity catalyze recent developments in biogenic AgNPs (bio-AgNPs) via biosynthesis, using natural mediators (plants, fungi, bacteria, and other organisms) that beneficially entail straightforward procedures, enhanced functions, and reduced ecotoxicity. On the surface, such benefits mitigate concerns around conventional AgNPs (Rai et al. 2021; Sati et al. 2025). Yet, high uncertainty remains over the extent to which biosynthesis is truly safe and sustainable, since numerous studies highlight the dire lack of precise ecotoxicity measurements of real-world effects of bio-AgNP exposure on organisms (Ottoni et al. 2019; Mussin and Giusiano 2022). Evidently, this broadly attempted "solution" against conventional AgNP ecotoxicity entails added problems.

Uncertainty over the "solution" necessitates further exploration of impacts of bio-AgNPs on life. Ecotoxicity investigations overwhelmingly show that bio-AgNPs entail lethal ecotoxicity for organisms and ecosystems. Specifically, numerous experiments conclude that bio-AgNPs

directly kill sensitive organisms, entailing immediately dire consequences. Moreover, biocascading effects—from deaths of sensitive organisms—reverberate through trophic structures, harming other species and undermining entire ecosystems (Vanlalveni et al. 2024; Tończyk et al. 2025). Specifically, for example, when bio-AgNPs kill soil microbes, destabilization of soil-based food webs inflicts devastating consequences on ecosystems (Ottoni et al. 2019). Evidently, biosynthesis—the perceived solution for AgNP ecotoxicity—adds new problems. Therefore—returning to original aims of environment-focused AgNP research to reduce ecotoxicity—this study aims to improve biosynthesis and make the "solution" an actual solution, not another problem. Reducing bio-AgNP ecotoxicity would entail benefits for human and environmental health to propel the world significantly closer to a truly safe and sustainable future.

Literature Review

AgNP Prevalence

As previously mentioned, AgNPs possess extremely high prevalence (Sati et al. 2025). Chemically-synthesized AgNPs are specifically most ubiquitous due to low-cost synthesis, with straightforward procedures attracting manufacturers. Thus, convenience of implementation (Quintero-Quiroz et al. 2019) causes chemical reduction to be the industry's most popular synthesis method (Nguyen et al. 2023; Nie et al. 2023). Meanwhile, demand for AgNPs increases consistently, since countless everyday items—textiles, sprays, bandages, toys, etc.—incorporate AgNPs into some part of their supply chain (Wei et al. 2014; Zheng et al. 2017; Nie et al. 2023; Shewale et al. 2025). Furthermore, recent trends predict already-rising demands for AgNP products (Hosny et al. 2025) to increase even more into the future (Nie et al. 2023). Consequently, increases in demand would elicit further increases in production, rendering AgNPs increasingly widespread and allowing dire issues to evoke reverberating impacts in today's world.

Ecotoxicity

Given overwhelmingly prevalent AgNPs pervading settings from labs and factories to hospitals and homes (Das and Paul 2025), urgent concerns arise over their proven ecotoxicity for living organisms (Harper et al. 2024; Hosny et al. 2025). Furthermore, AgNPs harm the environment, regardless of synthesis origin, particle structure, or ultimate function. Hence, the expansive spread of AgNPs couples with ecotoxicity to severely harm life on Earth, as numerous studies find particles—released by industrial manufacturing (León-Silva et al. 2016) and leached from everyday products (Das and Paul 2025)—entail lethal ecotoxicity (Tończyk et al. 2025). Further studies show manufacturing effluents (Lazim et al. 2023), wastewater discharges, and improper disposal acting as pathways (Ferdous and Nemmar 2020) releasing AgNPs into ecosystems to uncontrollably accumulate (Zheng et al. 2017). Ultimately, chemical pollution exponentially worsens over time, increasingly exposing organisms to lethal ecotoxicity. From

dead aquatic organisms to impaired soil-microbe communities (Tripathi et al. 2017), ecosystem structure and function (Toularoud et al. 2025) suffer from reduced biological activity (Waktole 2023). Therefore, overproduction and overconsumption of AgNPs entail overwhelmingly dire effects of intrusion into natural ecosystems, causing severe and irrevocable damage with lethal ecotoxicity.

Biosynthesis

In light of dire environmental implications of AgNPs, the aforementioned biosynthesis "solution" presents a somewhat-promising way forward, mitigating environmental detriments while still conveniently maintaining AgNP usage. Biosynthesis "connects" with nature (Madhusudanan et al. 2025), since organically-derived molecules reduce toxic ions back into original AgNPs (Eker et al. 2025). These natural molecules also serve as bio-enhanced capping agents that further optimize particle properties (Kamal et al. 2025). Due to perceived benefits, biosynthesis enjoys popularity as a realistic, sustainable solution, compensating for drawbacks of conventional synthesis. From investigating thermotolerant bacteria (Banerjee et al. 2025) to herbaceous creeper-plants (Ghosh et al. 2023), numerous studies enthusiastically explore biosynthesis. Evidently, researchers and stakeholders promote biosynthesis as pivotal for a simultaneously sustainable and profitable AgNP industry.

However, contrary to initial hopes, a new consensus now recognizes bio-AgNP ecotoxicity levels as comparable to their conventional counterparts. Tests on indicator organisms—specifically, Escherichia coli bacteria and Danio rerio zebrafish—show alarming ecotoxicity from seemingly innocuous bio-AgNPs, revealing potential to harm and even kill (Rheder et al. 2018). Hence, contrasting optimistic outlooks, bio-AgNPs remain far from "safe". Moreover, certain studies even suggest bio-AgNP ecotoxicity levels are higher than conventional ones. For example, when tested on Allium cepa cells, Swertia-leaf-derived bio-AgNPs confer similar—possibly higher—ecotoxicity compared to chemically synthesized AgNPs (Saha and Gupta 2017). Meanwhile, high concentrations of bio-AgNPs from Rumex-acetosa act via induced apoptosis mechanisms to undermine endothelial cell survival and zebrafish morphology, ultimately showing ecotoxicity levels greater than conventional AgNPs (Khan et al. 2019). Evidently, the perceived solution is another problem—an imperative issue plaguing the field of bio-AgNP research. Indeed, Bruni et al. (2024) found effective concentrations of biogenic and synthetic AgNPs to be 0.31μg/L and 0.01μg/L, respectively—meaning synthetic AgNPs were significantly less toxic than bio-AgNPs. Overall, most recent studies undeniably prove bio-AgNPs pose serious threats to safety and sustainability.

In light of these additional issues, various studies pursue ecotoxicity reductions by modifying the ongoing process of biosynthesis itself—emphasizing the "during-biosynthesis" stage. For example, Ali et al. (2025) mediate biosynthesis with Fusarium exquisite, hypothesizing that this ocean fungi, acting as the independent variable, "improves" biosynthesis. Similarly, Shaikhaldein et al. (2020) change only the specific organism—using Ochradenus arabicus, a different independent variable—tested as biosynthesis mediator. Continuing the trend,

Aboul-Nasr et al. (2025) also differ from aforementioned studies only by their specific biosynthesis mediator—kombucha cellulose nanocomposites—filling the role as yet another different independent variable. Evidently, endless possibilities exist for biosynthesis-mediating species, since any AgNP-processing organism may undergo testing as the valid independent variable "improving" standard biosynthesis. However, the commonality of these hundreds of studies lies in their fixation over optimizing the "ongoing" part of the intricate process. These studies explore improvements only within that "ongoing" process of directly synthesizing bio-AgNPs—thus lacking consideration of stages before and after, which include modification-based refinements of already-biosynthesized AgNPs. As a result of their overarchingly skewed focus, no universally effective approach currently exists for safe and sustainable biosynthesis.

However—despite generally misdirected attentions—certain studies do investigate modifications to processes outside of "ongoing" biosynthesis, with emphasis landing specifically on post-biosynthesis. da Silva Almeida (2025) successfully improved biosynthesis using cryoconcentration technology, finding superior colloidal stability and antimicrobial activity in bio-AgNPs that were modified after being fully biosynthesized. Their meaningful results contrast with ambiguously uncertain outcomes of most other post-biosynthesis studies. However, their biomedical priorities diverge from this study's environmental focus, which—combined with the fact that no studies adopt an environmental approach to explore post-biosynthesis ecotoxicity reduction—leaves substantial gaps to be filled in this field.

The current situation in bio-AgNP research elicits the question: how do post-biosynthesis modifications reduce bio-AgNP ecotoxicity, in order to render AgNP production and consumption more safe and sustainable? The researcher hypothesizes: generally, post-biosynthesis modifications significantly reduce bio-AgNP ecotoxicity, thereby presenting bio-AgNPs as a solution to truly promote safety and sustainability.

This study differs significantly from almost all other AgNP studies by accepting fully-biosynthesized bio-AgNPs from standard mediator organisms as they originally turn out—rather than attempting to improve the ongoing biosynthesis itself like almost all other studies do. Subsequently, the finished products of biosynthesis undergo separate post-synthesis modifications. In this study, a specific modification—thiol-based ligand exchange—replaces natural capping agents from biosynthesis with cysteine, a thiol containing an R-SH functional group, to enhance already-biosynthesized bio-AgNPs. This type of thiol-based modification has been shown—though not specifically with the inexpensive and accessible thiol cysteine or an environmental focus—to significantly improve the surface chemistry and stability of bio-AgNPs (Long et al. 2017). Consequently, this study adheres to typical practices for thiol-based ligand exchange, but specifically situates its investigation—featuring simplified, cysteine-based methods of ecotoxicity reduction—in larger goals of protecting the environment. This study is truly unique, as the only other recent study considering thiol-based ligand exchange was a calorimetric experiment analyzing effects of such modifications on enthalpy, entropy, and other thermochemical parameters (Huuppola et al. 2025). In parallel, the last experiment considering

thiol-based surface ligand exchange's effects in terms of bio-AgNP properties is relatively outdated, and retains a biomedical focus offering little insight for environmental sustainability (Johnston et al. 2016). Therefore, this environmentally-focused study—by prioritizing sustainability through pursuing ecotoxicity reduction—offers novel insight into future directions for bio-AgNP research and development, to yield a truly viable solution around safe and sustainable AgNPs in today's world.

Methods & Materials

In AgNP research, almost all studies anchor their methods in either experimentation or holistic analysis. Experiments range from testing AgNP ecotoxicity on marine life to exploring novel methods of biosynthesis with rare plants (Khan et al. 2019; Eker et al. 2025; Kamal et al. 2025). Evidently, experimentation represents the standard in AgNP research. Meanwhile, this study's unique exploration of post-biosynthesis modifications and environmental ecotoxicity necessitated a specific, tailored method, ultimately being primarily informed by Tończyk et al.'s ecotoxicity evaluation of Gloeophyllum-striatum-derived AgNPs (2025), tested on aquatic and soil organisms to demonstrate bio-AgNP ecotoxicity.

Safety & Growth Conditions

Aseptic technique—accepted and expected in microorganism-based experimentation—was followed (Bykowski and Stevenson 2020). An alcohol lamp was lit. Gloves were worn. Where applicable, cleaning agents were used. All equipment that needed to be sterilized was sterilized (dishes, flasks, loops, plates, etc.). These measures systematically reduced the likelihood of both the experiment and the experimenter being harmed.

Fusarium Growth & AgNP Biosynthesis

F.oxysporum was used in AgNP research as early as 2003: Ahmad et al. pioneered extracellular biosynthesis using this fungi as mediator. It additionally appears in studies that build on Ahmad et al.'s unprecedented study (Rahli et al. 2022; Tończyk et al. 2025). Evidently, F.oxysporum is a representative and reliable organism for biosynthesis mediation. Thus, this experiment selected F.oxysporum to enhance reliability and generalizability, and grew the fungi following standard procedure.

F.oxysporum and Potato Dextrose Agar (PDA) were purchased from Carolina Biological Supply Company. PDA was liquified, then poured to fill half the depth of a petri dish. After the PDA cooled, an inoculating loop gently picked small scoops of F.oxysporum from the agar slant edge and quickly streaked cultures across the PDA. The dish was sealed with parafilm and placed inverted into the incubator, at 27°C for five days.

4.85g of Potato Dextrose Broth (PDB) powder were weighed and added to 200mL of DI in a flask. The broth was stirred, boiled using a hot plate, autoclaved, and cooled by submerging in cold water. An inoculating loop was used to quickly and cleanly cut out a thin, circular F.oxysporum disk from the petri dish, transferring it whole into the now-cooled PDB, which was

sealed with parafilm and set to shake at 120 rpm in the shaker-incubator, at 27ºC. Three days later, the flask was removed from the shaker-incubator. Filter paper was placed over a funnel. The mixture was slowly poured through filter paper and funneled into another flask. 150mL of 5mM silver nitrate was added to 150mL of filtrate solution. The new 300mL solution was stirred, sealed with parafilm, and placed into the shaker-incubator at 150 rpm and 25-30ºC, for 24-72 hours in the dark. Later, successful bio-AgNP formation was visually confirmed by a color change from yellow to dark brown.

Bio-AgNP solution was pipetted from the flask into vials and centrifuged, first at 6000 rpm for 10 minutes and then at 10,000 rpm for 20 minutes. After, the supernatant in each vial—separate layers of clear, colorless liquid—was carefully and completely removed using a pipette, leaving solid brown pellets at the bottoms of vials. Finally, DI was added to each centrifuge vial to resuspend the pellets. The above procedure was repeated 5 times. After adding DI to resuspend pellets after the last repetition, all vials were stored at 4ºC.

Post-Biosynthesis Physicochemical Modifications

16 bio-AgNP vials were randomly assigned to be 8 experimental and 8 control groups. The experimental group underwent secondary centrifugation at 10,000 rpm for 20 minutes and subsequent cysteine modification. Cysteine—a thiol reasonable to obtain for high school research—was used for thiol-based surface ligand exchange, the most pivotal part of this experiment. The 8 control vials were taped to a beaker and held at room temperature. The centrifuged pellets were removed from their vials using a spatula, and suspended in 1% sodium dodecyl sulfate (SDS) solution that half-filled two test tubes. After thoroughly swirling, test tube caps were loosened to gently rest on the tubes, which were secured to a clamp. A beaker with water was placed over a hot plate. Test tubes were lowered into the beaker to boil for 30 minutes, then securely placed inside larger vials and centrifuged at 10,000 rpm for 20 minutes. Equal amounts of cysteine were added to each centrifuge vial, before incubating and shaking for four hours at 27ºC. The sample was then centrifuged at 10,000 rpm for 20 minutes.

E.Coli Growth, Bio-AgNP Exposure, & Area-Based Ecotoxicity Estimation

The use of E.coli as ecotoxicity indicators appears in various studies, with Dinc's study of ecotoxicity outcomes based on E.coli colony tests (2025) emerging most prominently. Specifically, their comprehensive toxicity assessment employed E.coli to evaluate harmful effects of conventional AgNP exposure, thereby differing from this study only in the type of AgNPs being investigated. After comparing with existing studies and evaluating compatibility, this study adopted E.coli as well, since widespread support exists—as previously shown—for selecting E.coli as indicator test organisms in experiments quantifying ecotoxicity effects on organisms. Similar to Dinc, Barani et al. (2021) also employ E.coli to test ecotoxicity. However, going one step further, they concretely justify their use of E.coli based on ecological reliability and accurate representations of effects of AgNP exposure on living organisms. Evidently, E.coli

are one of the best organisms for evaluating ecotoxic effects of AgNPs, both conventional and biosynthesized.

E.coli K-12 was purchased from Carolina. 50mL of Luria-Bertani (LB) broth was poured into a flask. The flask opening was stuffed with cotton before autoclaving at 121ºC for 30 minutes. After gently shaking the broth tube, an inoculating loop was quickly touched to the E.coli broth culture and then dipped into the autoclaved-and-cooled LB flask. The flask was gently swirled, sealed with parafilm, and placed into the incubator at 37°C for 12 hours. After, E.coli from the LB broth flask were poured to seep across the surface area of three prepoured LB Agar Plates from Carolina. Parafilm was wrapped around each of the three used plates, before incubating at 37 °C for 18 hours.

The three treatment groups in this experiment were: an experimental group testing post-biosynthesis physicochemically modified bio-AgNPs, a positive control group testing conventionally biosynthesized (unmodified) AgNPs, and a negative control group with E.coli grown under their standard, AgNP-free conditions. Any and all other parameters unrelated to AgNPs were kept as similar and as E.coli-friendly as possible, across all 3 groups.

Adding experimental groups to produce a gradient of bio-AgNP concentrations, both unmodified and modified, was unrealistic for a high school experiment with limited researcher skills and resources, tightly restrictive scheduling, and other school-setting-related limitations. Meanwhile, the lack of information available in the first place for this hyperspecific dimension of AgNP research also rendered some steps being taken to retain notable uncertainty. At certain times, entirely new measures—never explored in any other studies—had to be pioneered. Yet, despite a spectrum of bio-AgNP concentrations being the preferred design, this study's design of just three simple groups was also acceptable for AgNP research, as evidenced by authors such as Ashmore et al. employing a similar method, comparing "before" and "after" ecotoxicity on E.coli (2018), as well as Agnihotri et al., testing size-controlled particles in just three groups (2013). Therefore, though this experiment only used three groups, the decision to do so was intentional and effective.

AgNP solutions—regardless of type—must undergo dilution before usage in ecotoxicity tests on organisms, including the E.coli in this study. Without dilution, highly concentrated solutions eliminate all life forms immediately and completely, leading to losses of nuanced experimental outcomes (Aboul-Nasr et al. 2025). With this consideration, this study performed relative dilutions of 25% original AgNP solution to 75% DI for both unmodified and modified bio-AgNP treatment groups. This dilution method represented a compilation of multiple other methods' approaches to executing the exposure stage during ecotoxicity testing (Ashmore et al. 2018; Ghosh et al. 2023; Ali et al. 2025). Ultimately, equal volumes of unmodified and modified bio-AgNPs were added to equal volumes of DI in two flasks.

Three autoclaved pieces of filter paper were resized to fit the petri dishes, each dipped into one of three solutions (DI, unmodified bio-AgNPs, or modified bio-AgNPs), and carefully and smoothly laid on top of E.coli in plates. The treated petri dishes were placed into the incubator at 37°C.

1 day later, E.coli survival was measured using the Kirby-Bauer test, approximating remaining colony areas in each plate after a day of treatment, as a school-friendly way of evaluating minimum inhibitory concentration, the standardly employed parameter that entails more complicated measurements (Vanlalveni et al. 2024; Tończyk et al. 2025). A smaller area meant greater ecotoxicity for E.coli. A larger area meant less. The final area ranking was expected to be: unmodified < modified < control, which would suggest modified bio-AgNPs to be less toxic than unmodified ones.

Results

Colony Area

Figure 6 shows area approximations and calculations for unmodified ("UNMOD") and modified ("MOD") bio-AgNP E.coli treatment groups. Ignoring colony density differences—a decision that ultimately proved to be quite problematic—between groups, the negative control group area (equal to the total area of each petri dish) was calculated with: $\pi\bullet(8.50/2)^2 = 56.745\text{cm}^2$

The positive control group area was 36.727cm^2, calculated by subtracting the sector area without E.coli from the total area. The experimental group area was 11.664cm^2, calculated by adding the three component sector areas with E.coli. Note that all sector areas shown were calculated with: (sector angle)/360=(sector area)/(total area), with sector angles measured using a protractor. Figure 7 organizes each treatment's colony areas, and provides additional notes regarding the measurements and data.

Kruskal-Wallis Test

Statistical options—from t-tests to ANOVA—were first evaluated from a purely functional perspective, in order to find a test that could uniquely align with this experiment's novel nature, especially in how school-specific conditions led the data to comprise just three area values. Ultimately, the Kruskal-Wallis test was selected from Mirhashemi et al.'s study comparing not-normally-distributed data values, since this experiment's three area values were similar in nature to its post-AgNP-exposure microorganism colony size measurements (2021). Indeed, despite Mirhashemi et al. testing types of AgNPs unrelated to biosynthesis on microorganisms different from E.coli, the general structure of their investigation aligned well enough with this study, justifying adoption of their statistical method.

Performing the Kruskal-Wallis test, each area value was treated as a single sample and ranked from smallest (1) to largest (3) (see table above). Ranks were plugged into the formula:

$$H = 12/(N(N+1))\Sigma R_i^2/n_i - 3(N+1)$$
$$\Sigma R_i^2/n_i = (9/1) + (4/1) + (1/1) = 14$$
$$\text{constant} = 12/(3 \times 4) = 1$$
$$\text{H-Statistic} = (1 \times 14) - 3(4) = 14 - 12 = 2$$

The H-Statistic (H=2) was found to be less than the chi-square value for two degrees of freedom (χ^2=5.99): 2 < 5.99. The p-value was found to be greater than the significance level (α=0.05): 0.368 > 0.05. Therefore, the difference was concluded to be not statistically significant.

Discussion

General Conclusions

Contrary to the researcher's original hypothesis, this experiment yielded absolutely no statistically significant difference between numerical values for final E.coli colony areas after three separate exposures to DI, unmodified bio-AgNPs, and modified bio-AgNPs. In effect, this study fails to enhance the understanding of this field of research regarding the effectiveness of post-biosynthesis physicochemical modifications in reducing bio-AgNP ecotoxicity.

In this experiment, colony areas served as indicators of ecotoxicity, due to the fact that their values depended almost completely on E.coli death and survival in each colony—a dependent variable that in turn depended almost completely on the specific treatment exposure. Given that no statistically significant difference was found between the final colony areas of 56.745cm^2, 36.727cm^2, and 11.664cm^2, one concludes no statistically significant difference in E.coli survival across the three different exposure treatments. Thus, no difference exists between the three ecotoxicity levels entailed by the three treatments—modified bio-AgNPs, unmodified bio-AgNPs, and DI (no AgNPs). Indeed, the study fails to conclude that modified bio-AgNPs entail significantly reduced ecotoxicity. In other words, modifications of bio-AgNPs with post-biosynthesis thiol-based ligand exchange fail to reduce ecotoxicity, contrary to the initial hypothesis that such modifications would reduce ecotoxicity to such a significant extent that bio-AgNPs could once again offer hope as a safe and sustainable solution.

Specific Observations

Although ultimately, quantitative results lacked statistical significance (p > α=0.05), supplementary qualitative observations of high ecotoxicity in modified bio-AgNPs suggests a key finding: post-biosynthesis modifications may, in fact, be insufficient to counteract inherently ecotoxic bio-AgNPs. Indeed, biosynthesis appears to not inherently alter the long-term environmentally detrimental behavior of AgNPs. Therefore, the focus of this field of research may benefit from a shift from surface-level modifications to profound alterations to the practice of biosynthesis itself, or even to the entire production line for AgNPs in general, in order to create truly safe and sustainable bio-AgNPs. Indeed, this study brings the research back to the original concerns raised by studies showing that bio-AgNPs, contrary to their endorsers' beliefs, do still entail substantial issues in terms of ecotoxicity (Bruni et al. 2024; Tończyk et al. 2025).

Meanwhile, this experiment—direly demonstrating E.coli deaths from not one but two types of bio-AgNP exposure—adds evidence corroborating conclusions from numerous studies showing ecotoxic bio-AgNPs harming living organisms (Ottoni et al. 2019). Specifically, corroborating Rheder et al.'s zebrafish tests (2018), E.coli deaths in this experiment do indeed

prove the dire ecotoxicity of seemingly innocuous bio-AgNPs. Any aforementioned uncertainty regarding the increasingly prominent drawbacks of biosynthesis is thus further reduced in the face of this experiment adding more evidence that parallels the facts that already exist.

Furthermore—though keeping in mind the lack of statistically significant differences between area values—it should be noted that the final area ranking turned out as: modified < unmodified < control, which contradicts previous predictions, as modified bio-AgNPs now appear to actually be even more harmful than unmodified bio-AgNPs. In a broad sense, this observation would support Saha and Gupta's conclusion that leaf-derived bio-AgNPs confer higher ecotoxicity than conventional AgNPs (2017). Meanwhile, such a result would also corroborate Bruni et al.'s findings of synthetic AgNPs turning out to be less harmful than bio-AgNPs (2024). However, recalling that the earlier Kruskal-Wallis test renders current numerical comparisons to lack statistical significance, this observation—though theoretically intriguing—lacks concrete experimental support.

The unique approach of this experiment starkly contrasts the nature of almost all other known studies, in terms of purpose, process, and numerous other key elements. Only specific steps occasionally needed to overlap with currently known work in the field, in order to ensure certain outcomes that would allow the experiment to continue smoothly. Nonetheless, as previously described, this experiment refused to fixate on changing the convoluted ongoing biosynthesis process like Ali et al. (2025) and countless others attempted. Instead, it adopted the post-synthesis AgNP-modification methods of Huuppola et al.'s thermochemical investigation (2025) and Johnston et al.'s biomedical evaluation (2016), coupling their rare, novel approaches with a meaningful environmental focus to strive to address increasingly dire bio-AgNP ecotoxicity. The unprecedented nature and focus of this experiment—despite the insignificant results—thus allow the study to retain significant value.

Overall, this experiment admittedly offers no groundbreaking discoveries around the ability of physicochemical post-biosynthesis modifications—specifically, thiol-based ligand exchange with the chemical cysteine—to significantly reduce bio-AgNP ecotoxicity and improve AgNP production and consumption to be more safe and sustainable. This experiment can only confidently conclude that its specific investigated modifications fail to meaningfully reduce bio-AgNP ecotoxicity. A truly safe and sustainable solution to this dimension of chemical pollution in today's world is thus still waiting to be found.

Conclusion

Driven by the urgent need to protect human and environmental health, this study investigated whether post-biosynthesis physicochemical modifications could successfully reduce bio-AgNP ecotoxicity. Specifically, the efficacy of post-biosynthesis thiol-based ligand exchange was explored using a common modifier—the amino acid cysteine—to alter bio-AgNP surface chemistry. Utilizing E.coli as an indicator organism, the experiment ultimately revealed that the specific cysteine-based modification failed to reduce bio-AgNP ecotoxicity. Although colony area approximations paradoxically indicated that modified particles were slightly more lethal

than their unmodified counterparts, a Kruskal-Wallis test determined these differences lacked statistical significance. Consequently, the study concludes this specific post-biosynthesis modification fails to yield conclusive ecotoxicity reduction.

The significance of these findings lies in their direct confrontation of preexisting assumptions that biosynthesis inherently produces safe alternatives to conventional AgNPs. By confirming that bio-AgNPs retain profoundly lethal ecotoxicity for indicator species, this study highlights biosynthesis as a deceptive, incomplete solution, entailing pivotal implications for theory, practice, and policy. Theoretically, it addresses a significant gap in AgNP research: while 99% of existing studies hyperfixate on optimizing parameters during active biosynthesis, this study shifts focus toward the overlooked post-biosynthesis stage and situates thiol-based ligand exchange within an environmental sustainability context, not biomedical or thermochemical. In practice and policy, the results represent a warning to stakeholders that bio-AgNPs cannot be recklessly adopted as an ideal solution guaranteeing safety and sustainability, contrary to initial enthusiasm.

These conclusions should be interpreted alongside major limitations inherent in a high-school laboratory setting. Due to flaws in scheduling, resources, and skills, the design was constrained to a single dilution instead of an overarching concentration gradient, and the area estimation problematically overlooked variations in colony density. Furthermore, time barriers prevented execution of another identical trial or simultaneous parallel experiments, limiting the amount of data and restricting statistical power for detecting nuanced relationships.

To advance this significant field of environmental science and engineering, future research should adhere more strictly to aseptic techniques to bolster data validity, establish concentration gradients to track dose-dependent effects, and integrate multiple trials to reconcile operational errors and improve statistical significance. Beyond cysteine, alternative low-cost thiols and post-synthesis modification mechanisms await exploration to discover the ideal improvement method. Ultimately, although this study failed to offer a revolutionary solution for ecotoxicity reduction, its value remains high as a pioneering investigation. By eliminating an ineffective possibility today, this study represents a critical stepping stone bringing this field of research closer to an authentically safe and sustainable solution for AgNP pollution tomorrow.

Tables And Figures

Figure 1

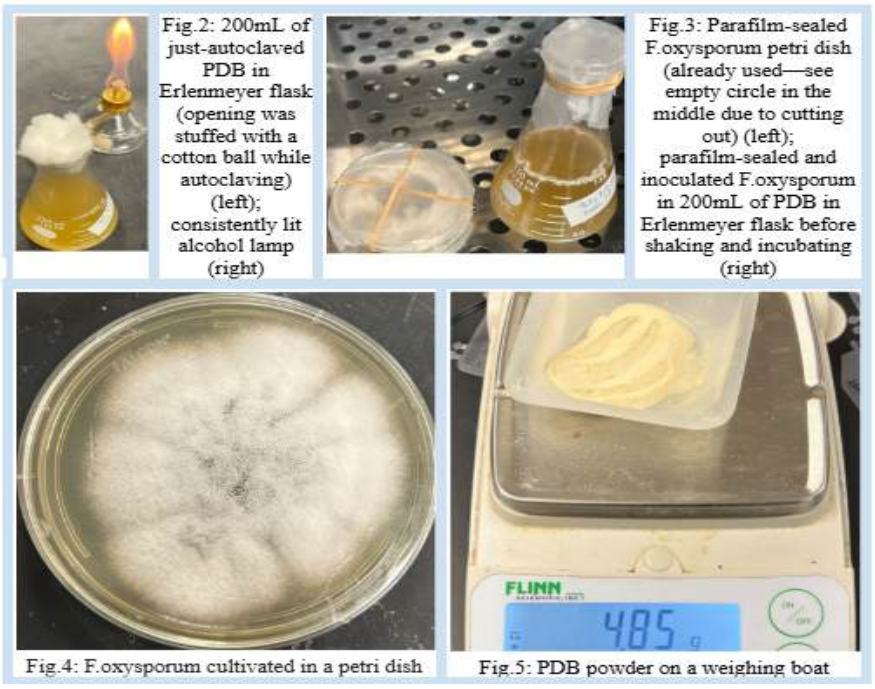


Figure 2

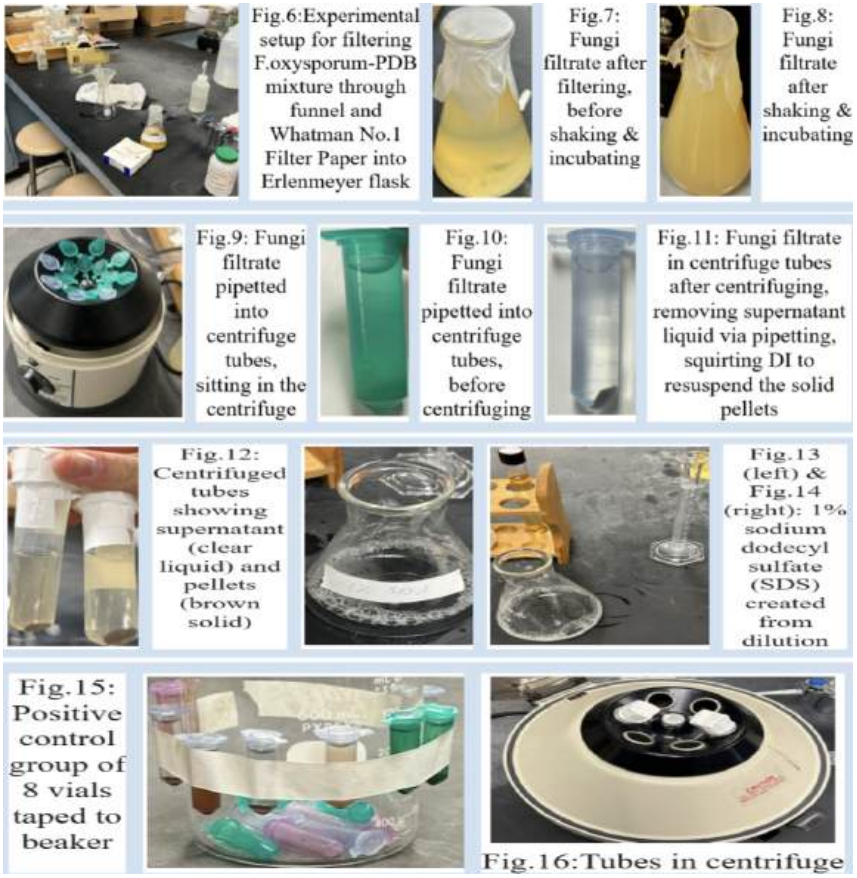


Figure 3

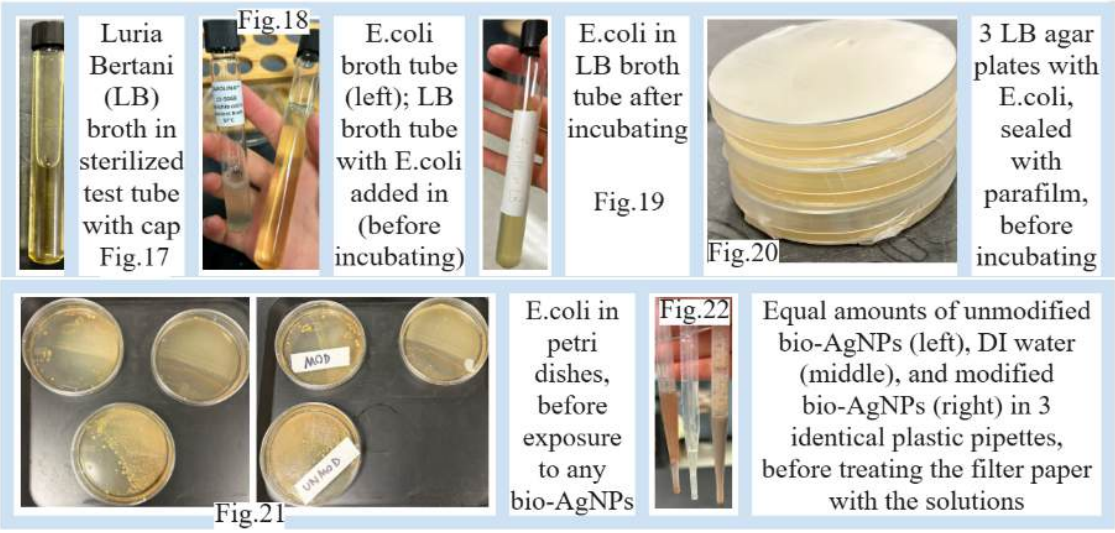


Figure 4

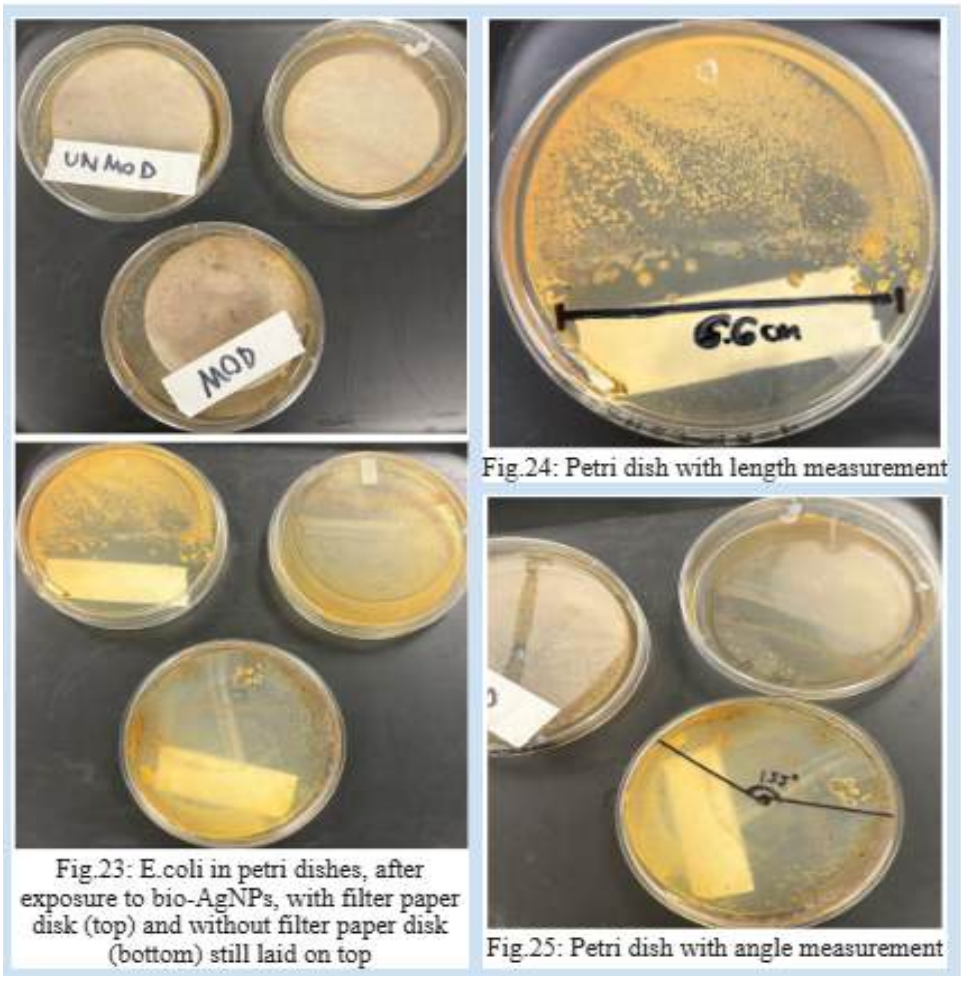


Figure 5

Treatment	Approximate Diameter (cm)	Calculated Area (cm^2)	Notes
(-) Control (No AgNPs)	8.50 cm	56.745 cm^2	- Although area *is* calculated, this value ignores visible colony density differences
(+) Control (Unmodified Bio-AgNPs)	See notes	36.727 cm^2	- less (but still notable) density differences entail the same explanation as 1 - method of area calculation was changed after observing results; forced to pivot from using diameter (turned out unmeasurable/nonexistent) to using sector areas calculated by ratios. Therefore, no approximate diameter recorded/used
Experimental (Modified Bio-AgNPs)		11.664 cm^2	

Figure 6

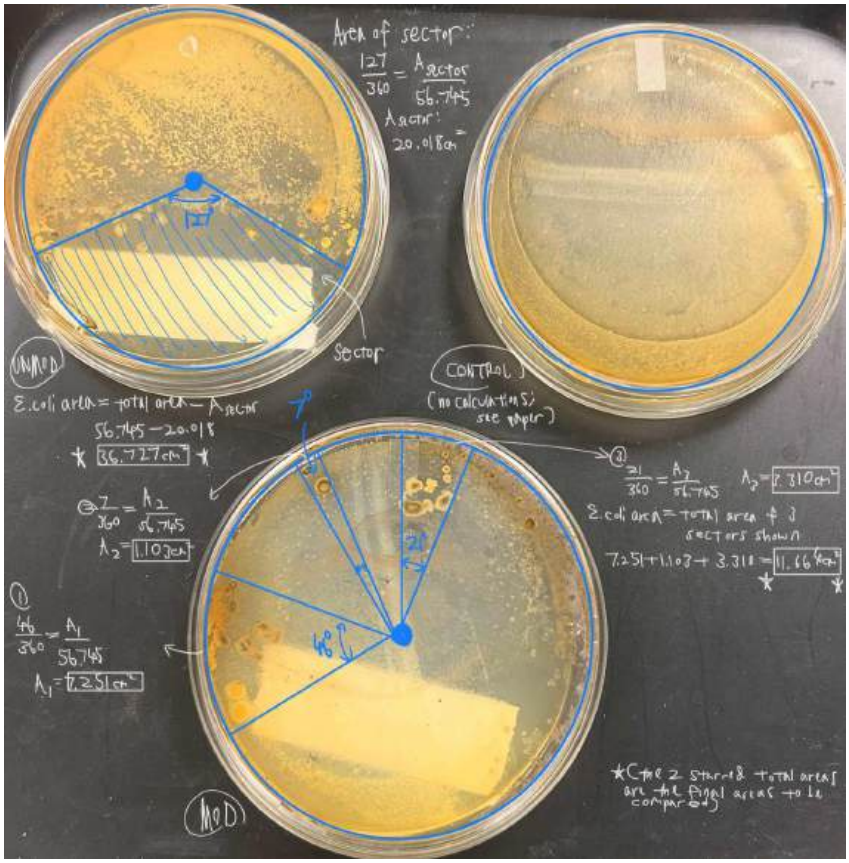


Figure 7

Group	Value	Rank (R_i)	Rank Squared (R_i^2)
Group 1	56.745	3	9
Group 2	36.727	2	4
Group 3	11.664	1	1
Total (N)	**3**	**Sum**: 6	**Sum (R_i^2)**: 14

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Association Between Self-Reported Academic Stress and Hair Loss/Excessive Shedding Among High School Students By Fiona Catherine Antony Sudharsan

Abstract

High school students commonly deal with academic stress, and that stress doesn't always stay in the mind, it can show up physically too. This study "Association Between Self-Reported Academic Stress and Hair Loss/Excessive Shedding Among High School Students" looked into whether there is a connection between self-reported academic stress and hair loss or excessive shedding, using a cross-sectional survey of 250 students in grades 9–12. Out of the 250 students, 97 (38.8%) said they experienced hair loss or excessive shedding, while a much larger group, 212 students (84.8%), reported high or very high academic stress. As stress categories increased, so did the prevalence of hair loss, reaching 45.8% among students who reported very high stress. Students who reported high or very high stress had about four times the odds of also reporting hair loss or excessive shedding compared to students who reported very low, low, or moderate stress (OR 4.01, 95% CI 1.61–10.00, p = .0018), meaning the relationship is unlikely to be due to chance alone. Academic stress also showed a positive ordinal association with the hair-loss outcome (Spearman ρ = .205, p = .0011), so as stress level went up, so did the tendency to report hair loss. Even after adjusting for other factors in an exploratory model, each one-category increase in academic stress was still associated with greater odds of reporting hair loss or excessive shedding (adjusted OR 1.77, 95% CI 1.17–2.69, p = .0068). Altogether, these findings support an association between academic stress and self-reported hair changes, but they don't prove that stress causes hair loss, and they can't confirm an actual clinical diagnosis of telogen effluvium.

Keywords: academic stress; adolescents; hair shedding; hair loss; sleep; high school students

Introduction

Academic demands like examinations, homework, extracurricular commitments, and planning for the future can all add up to create stress during adolescence. Research done on secondary-school populations has linked this academic stress to sleep and psychological health, which suggests that school-related stress might be connected to a student's overall well-being, not just their mental state (Zhang, Wang, and Zhang; "Sleep Quality").

Hair follicles naturally cycle through phases of growth, transition, resting, and shedding. One recognized form of hair shedding is telogen effluvium, which is diffuse (spread out) and non-scarring, and it can be triggered by several things, including physiological and emotional stress (Jimeno Ortega and Stefanato; Yin et al.; Asghar et al.). However, telogen effluvium is technically a clinical diagnosis, meaning it has to be confirmed by a doctor, so it can't be established just through an anonymous questionnaire. Because of this, the study evaluates self-reported hair loss or excessive shedding rather than a diagnosed case of telogen effluvium.

The main research question was whether higher self-reported academic stress is associated with a greater likelihood of self-reported hair loss or excessive shedding among high school students. Our hypothesis was that students who reported higher academic stress would also be more likely to report hair loss or excessive shedding.

Methods

Study Design and Participants

This analysis included 250 completed survey responses from students in grades 9–12. Each student was de-identified and given an ID from S001 to S250, so no individual answers could be traced back to a name. Out of the 19 variables that were analyzed, there were no blank values anywhere in the dataset, which made the analysis more reliable. The survey covered a range of topics: academic workload, academic stress, how often that stress occurred, physical or emotional stress symptoms, sleep, hair loss and shedding, family history, nutrition, physical activity, how well students coped with stress, and how they perceived the effects of stress and hair changes.

Variables

The main exposure being measured was self-reported academic stress level, which had five ordered categories: very low, low, moderate, high, and very high. The main outcome was a simple yes or no response to whether the student experienced hair loss or excessive shedding. On top of that, secondary measures were also collected, including how often hair loss occurred, whether hair shedding had changed, and whether hair thinning was visible. The rest of the variables were looked at descriptively or used as exploratory covariates (meaning extra factors that could help explain the results).

Statistical Analysis

For the categorical variables, counts and percentages were calculated. Academic stress was coded on an ordinal scale from 1 (very low) to 5 (very high), and a Spearman rank correlation was used to look at these ordinal relationships. To simplify things further, a two-by-two comparison was also done, combining high/very high stress versus very low/low/moderate stress, and from that an odds ratio was estimated along with a 95% confidence interval; Fisher's exact test was used to get the corresponding p value. Continuous variables were compared between groups using Mann–Whitney U tests. Additionally, an exploratory multivariable logistic regression was used to model hair loss or excessive shedding while adjusting for study hours, homework hours, sleep duration, family-history indicators, nutrition quality, physical activity, and coping effectiveness. A two-sided p value of less than .05 was considered statistically significant.

Results

Participant Characteristics

Characteristic	Result
Participants	250
Grade 9	59 (23.6%)
Grade 10	60 (24.0%)
Grade 11	73 (29.2%)
Grade 12	58 (23.2%)
Female	128 (51.2%)
Male	114 (45.6%)
Other/prefer not to say	8 (3.2%)
Mean study hours/day	3.22 ± 1.25
Mean homework hours/day	2.00 ± 0.85
Mean sleep hours/night	6.31 ± 0.79

Academic Stress and Hair-Loss Prevalence

High or very high academic stress was reported by 212 out of the 250 students, which comes out to 84.8%. Hair loss or excessive shedding, meanwhile, was reported by 97 students (38.8%). As shown below, the prevalence of the hair-loss outcome kept rising as the stress category increased.

Academic stress level	Students	Hair loss/shedding: Yes	Prevalence
Very low	3	0	0.0%
Low	8	1	12.5%
Moderate	27	5	18.5%
High	59	21	35.6%
Very high	153	70	45.8%

This association across the five stress categories was statistically significant ($\chi^2 = 12.28$, df = 4, p = .0154), although it's worth noting that the two lowest stress groups only had a small number of students in them. The ordinal relationship between academic stress and the hair-loss outcome (yes/no) was positive (Spearman $\rho = .205$, p = .0011), meaning that as stress increased, so did the tendency toward hair loss. When stress was grouped into just two categories, high/very high versus very low/low/moderate, hair loss or excessive shedding occurred in 91 of the 212 students (42.9%) in the higher-stress group, compared to only 6 of 38 students (15.8%) in the lower-stress group. This gave an odds ratio of 4.01 (95% CI 1.61–10.00; Fisher p = .0018).

Secondary Findings

Academic stress was also positively associated with how much students said their hair shedding had changed (Spearman $\rho = .202$, p = .0013), and it was inversely associated with sleep

duration (Spearman $\rho = -.378$, $p < .001$), meaning that as stress went up, sleep tended to go down. Students who reported stress-related physical or emotional symptoms had a hair-loss/shedding prevalence of 45.3%, which was noticeably higher than the 27.0% seen among students who didn't report those symptoms (OR 2.25, 95% CI 1.28–3.94, $p = .0045$). The mean study time was 3.40 hours per day for students who reported hair loss or shedding, compared to 3.10 hours for those who didn't ($p = .055$). Similarly, mean homework time was 2.11 versus 1.93 hours per day ($p = .095$), and mean sleep duration was 6.22 versus 6.37 hours per night ($p = .246$). None of these group differences reached statistical significance at the .05 level, so they should be interpreted cautiously.

Exploratory Adjusted Model

In the exploratory multivariable logistic regression, each one-category increase in academic stress was associated with 1.77 times the odds of reporting hair loss or excessive shedding, even after adjusting for the selected covariates (95% CI 1.17–2.69, $p = .0068$). That said, since this study wasn't specifically designed to estimate the effects of every covariate, this adjusted model should be treated as exploratory rather than conclusive.

Discussion

The main finding of this study was a statistically significant association between higher self-reported academic stress and self-reported hair loss or excessive shedding. The prevalence of the hair-loss outcome climbed from 18.5% among students reporting moderate stress, to 35.6% among those reporting high stress, up to 45.8% among those reporting very high stress. In other words, students in the high/very high stress group had roughly four times greater odds of reporting hair loss or excessive shedding compared to students in the combined lower-stress group.

This pattern lines up with clinical literature that identifies emotional and physiological stress as possible triggers of telogen effluvium and other forms of hair shedding (Jimeno Ortega and Stefanato; Yin et al.; Asghar et al.). Even so, this survey can't determine an actual diagnosis or the biological mechanism behind it. Hair loss can also be influenced by plenty of other things, illness, nutrition, endocrine conditions, medications, genetics, and hair-care practices, none of which were comprehensively measured in this survey.

The inverse relationship between academic stress and sleep duration is also worth pointing out here. Higher stress tended to go along with fewer hours of sleep, although sleep duration on its own didn't significantly differ between the two hair-loss groups. And because this study was cross-sectional (meaning everything was measured at one point in time), the direction of these relationships, meaning what's actually causing what, can't be established.

Limitations

There are several important limitations to keep in mind. Both the exposure and outcome were self-reported, there wasn't a validated multi-item academic-stress scale used, no clinical

dermatologic examination was done, and the design was cross-sectional. There's also the possibility of selection bias, the lowest stress categories only had small numbers of students, and confounders like medical, hormonal, nutritional, and hair-care factors weren't fully measured. On top of that, the hair-loss variables in the dataset were very closely linked to each other structurally, which is probably because of how the questionnaire branched, so they shouldn't be treated as independent confirmations of the main outcome. Overall, these findings only demonstrate an association, they should not be taken as evidence that academic stress actually causes telogen effluvium.

Conclusion

Among the 250 high school students in this study, higher self-reported academic stress was associated with a greater likelihood of self-reported hair loss or excessive shedding. High or very high stress was associated with roughly four times the odds of the hair-loss outcome compared to the lower stress categories, and this association remained statistically significant even in the exploratory adjusted model. Moving forward, future research should use validated stress instruments, objective dermatologic assessments, and more thorough measurement of potential confounders to build on these findings.

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Works Cited

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