***NIH R21 Grant Proposal:*** *NIH Exploratory/Developmental Research Grant Program (Parent R21 Clinical Trial Not Allowed)* *PA-25-304*

***Title:*** Investigating the Relation between Iron Deficiency, Hippocampal Volume, and Obesity Risk: A Conceptual Analysis Using the NIH ABCD Dataset

***Project Summary (30 lines):***

Obesity and iron deficiency (ID) are two widespread yet distinct public health crises, both with an alarming prevalence among adolescents worldwide. Obesity, which has quadrupled among children and adolescents since 1990, is a major risk factor for chronic diseases such as diabetes, cardiovascular disease, and hypertension, as well as reduced health-related quality of life due to social stigma and psychological distress. Simultaneously, ID, the most common nutritional deficiency, affects up to 21% of adolescents globally, impairing cognitive function and overall well-being. Emerging research suggests an intersection between obesity and ID; however, a significant gap in knowledge remains regarding the mechanisms linking them.

During adolescence, hormonal changes, increasingly independent food choices, and physical activity influence obesity risk, while rapid growth and menstruation elevate iron demands, making ID prevalent. Disrupted iron homeostasis during this stage impacts multiple organs, particularly the brain.

We propose the volume of the hippocampus, a brain region critical for learning and memory, as a novel mediator in the relation between ID and obesity risk. Iron is essential for hippocampal neuronal development and synaptic function, and deficiency of iron has been associated with lower hippocampal volume. Preclinical studies demonstrate that lower hippocampal volume is associated with dysregulated appetite and increased obesity risk. Based on this, we hypothesize that ID may be related to obesity risk due to its effect on the hippocampus.

We will utilize data from the NIH Adolescent Brain Cognitive Development (ABCD) study —the largest long-term study of brain development and child health in the U.S., following nearly 12,000 children from ages 9-10 to 19-20. We will investigate the potential novel pathway between ID, hippocampus, and obesity through three specific aims:

**Aim 1:**Investigate the relation between iron status and hippocampal subregion/subfield volumes, specifically the cornu ammonis regions (i.e. CA1, CA2, CA3, and CA4), dentate gyrus, and the subiculum.

**Aim 2:** Examine the association between iron status and anthropometric measures using body mass index and waist circumference (BMI-WC)

**Aim 3:** Investigate hippocampal volumes as mediators between ID and higher BMI-WC

Due to the scarcity of established frameworks and empirical data to guide our hypothesis, leveraging the ABCD dataset for secondary analysis is both strategic and efficient. This approach will allow us to explore an under-researched area while minimizing the resource demands and risks of launching a new clinical trial. The insights gained could significantly advance our understanding of obesity prevention and management, potentially transforming clinical practices and public health strategies for individuals at risk of obesity and ID.

***Project Narrative (3 sentences):*** This research seeks to reveal how iron deficiency may impact brain structure, potentially leading to overeating and obesity in adolescents. Insights gained from this study could inform new strategies for preventing and managing obesity, ultimately enhancing the health and well-being of young people and reducing the risk of related diseases in the future.

***Specific Aims (1 page)***

**Background**

Obesity and iron deficiency (ID) are two widespread yet distinct public health crises, both with an alarmingly high prevalence among adolescents worldwide. As of 2022, over 390 million adolescents aged 5–19 were overweight, and 160 million lived with obesity—conditions tied to chronic diseases and health related quality of life (1). Simultaneously, ID, the most common nutritional deficiency, affects up to 21% of adolescents globally, impairing cognitive function and overall well-being (2). While these conditions are typically studied in isolation, emerging evidence suggests they may be interconnected in ways that are not yet fully understood (3). Adolescence is a period of rapid growth, puberty, and brain development which increases iron requirements and makes ID especially prevalent (2,4). Among brain regions, the hippocampus is particularly vulnerable to ID due to its high metabolic demands and reliance on iron for neurogenesis, myelination, and synaptic development (5). Research shows that lower iron levels during adolescence are linked to poorer white matter fiber integrity years later, potentially impairing long term neural connectivity and cognitive function (6,7). Furthermore, ID disrupts hippocampal structure, which is critical for regulating food intake and appetite, achieved through the integration of mnemonic, sensory, and interoceptive information (8–12). Therefore, structural impairments in the hippocampus may undermine its ability to regulate eating behavior, potentially contributing to overeating and an increased risk of obesity (13–15). This study represents a paradigm shift in understanding adolescent obesity by identifying lower hippocampal volume as a novel neurobiological mechanism linking ID to obesity risk.

**Hypothesis**

We hypothesize that ID reduces hippocampal volume, which disrupts appetite regulation mechanisms that increase susceptibility to overeating and obesity. This hypothesis emerges from two sets of empirical evidence: 1. ID significantly impairs hippocampal development, affecting neuronal architecture and synaptic plasticity, and (8), 2. compromised hippocampal structure directly disrupts appetite regulation by impairing meal-related memory and increasing responsiveness to food cues (13). Using the NIH Adolescent Brain Cognitive Development (ABCD) dataset, we will systematically investigate if Hippocampal volume in ID adolescents is related to BMI-WC, through the following specific aims:

***Specific aim 1: Investigate the relation between iron status and hippocampal subregion and subfield volumes in adolescents***

Using available serum iron biomarkers within the ABCD dataset (hemoglobin, ferritin) and available structural MRI data, we will analyze how variations in iron status correlate with hippocampal subregion volumes (the cornu ammonis regions i.e. CA1, CA2, CA3, and CA4, dentate gyrus, and the subiculum) while accounting for demographic, developmental, physiological, psychosocial, and neuroanatomical factors. We hypothesize that adolescents with ID will exhibit lower hippocampal subregion and subfield volumes (CA1, CA2, CA3, CA4, dentate gyrus, and the subiculum) over time compared to adolescents without ID.

***Specific aim 2: Examine the association between iron status and body mass index and waist circumference (BMI-WC) in adolescents***

Using available serum iron biomarkers within the ABCD dataset (hemoglobin, ferritin) and available anthropometric measures of body mass index and waist circumference, we will analyze how iron status is related to BMI-WC. We hypothesize that adolescents with ID will have higher BMI z-scores, and waist circumference compared to adolescents without ID.

***Specific aim 3: Investigate Hippocampal volume as a mediator between iron deficiency and BMI-WC.***

We will conduct a mediation analysis to quantify indirect effects of iron status on BMI-WC through hippocampal volume. We hypothesize that Hippocampal volume will mediate the relation between ID and BMI-WC (lower hippocampal volume being related to higher BMI-WC), suggesting a neurobiological mechanistic link between iron status and obesity.

**Impact**  
This innovative study will identify whether ID-related changes in hippocampal volume contribute to obesity risk, providing the first human evidence of a link between iron status and obesity through brain alterations. Success could inform continued research to transform prevention strategies, including iron supplementation—a simple, low-cost intervention—offering a powerful tool to reduce obesity risk. Early intervention could prevent irreversible hippocampal damage, safeguarding long-term cognitive and metabolic health. This research has the potential to reshape public health approaches, offering scalable solutions to reduce overweight and obesity rates while simultaneously addressing neurocognitive challenges in youth.

***Research Strategy****(6 pages)*

***Significance:***

Obesity is a complex, multifactorial, and preventable public health crisis, affecting over a third of the global population, including over 390 million children and adolescents as of 2022. Since 1990, rates have more than doubled among adults and quadrupled among children and adolescents, with projections indicating that by 2030, 38% of adults will be overweight and 20% will have obesity (1,16,17). The persistently high prevalence despite numerous prevention efforts emphasizes the urgent need for more research aimed at understanding its underlying drivers. Obesity during childhood and adolescence significantly increases the risk of numerous health problems, including subsequent cardiovascular diseases, type 2 diabetes, and mental health disorders such as anxiety and depression (16–18).

Concurrently, iron deficiency (ID) remains the most prevalent and preventable single nutrient deficiency globally, recognized by the World Health Organization (WHO) as a significant global health concern (2,19,20). At the global level, the prevalence of adolescent anemia, of which 50% is attributed to iron deficiency (21), is estimated at 15%, but with large disparities between high and low-middle income countries (6% and 27%, respectively) (22,23). Anemia is most common during infancy and early childhood but remains highly prevalent during adolescence (2,24). Of concern, ID without anemia, often referred to as non-anemic iron deficiency (NAID), is even more common and frequently overlooked. Studies suggest that NAID could be up to twice as prevalent as iron deficiency anemia (IDA), affecting a substantial proportion of adolescents (25,26). Even in the US, research indicates that nearly 40% of adolescent girls and young women may have low iron levels, with many cases being non-anemic (27). The impact of ID extends beyond hematological manifestations, affecting cognitive, emotional, and behavioral development. More specifically, iron-deficient children exhibit reduced mental and motor development, alongside behavioral alterations (7, 20–22) while adolescents with ID and iron deficiency anemia (IDA) experience lower quality of life tied to fatigue, poor academic performance linked to attention deficits and memory issues, poor emotional regulation, heightened anxiety levels, and reduced psychosocial well-being (31).

A key factor in the cognitive and emotional consequences of iron deficiency is iron’s role in brain health, particularly its critical function in the hippocampus— a brain region involved in learning and memory. Iron plays a crucial role in neuronal maturation in the hippocampus, including axon and dendrite growth, synapse formation and maintenance, mitochondrial energy production essential for dendritic arborization, and neurotransmitter balance and synaptic protein expression (5,9,32). When deprived of iron, hippocampal neurons face an energy crisis, impairing their ability to form dendritic arbors (Figure 1) (33). A reduction in dendritic complexity signifies compromised neuronal architecture, potentially leading to long-term impairments in synaptic function and efficacy, negatively affecting learning and memory (8–10,32). Alarmingly, even without the presence of anemia, ID can cause significant structural and functional impairments in the hippocampus that may lead to irreversible damage (5), emphasizing the importance of proper iron status, especially during development.

A collage of images of neurons

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*Figure 1: ID impairs the dendritic arbor complexity of cultured hippocampal neurons. Representative stage 1 (A) and stage 2(B) images of neurons and tracings [primary dendrite (red/black), secondary branch (blue/dark gray), tertiary branch (green/light gray)] of control (Aa, Ac, Ba, Bc) and ID (Ab, Ad, Bb, Bd) neurons. {Figure extracted from Carlson et al. (2009)}*

The hippocampus not only plays a critical role in memory and cognitive processes but also significantly influences eating behavior by maintaining memories of meals. Forming memories of meals contributes to appetite management and delays the onset of subsequent meals, thereby playing a crucial role in preventing overeating, which can lead to overweight and obesity (34). The ventral hippocampus (vHP) serves as a critical integrator of feeding-related signals by processing meal-related memories, food-associated cues, sensory information, and metabolic signals (e.g., leptin, insulin, ghrelin). Both severe disruptions (e.g., amnesia) and more subtle impairments in hippocampal function—such as those associated with stress, or metabolic dysregulation—can lead to impaired meal memory and heightened responsiveness to food cues (35,36). These disruptions may create a cycle that promotes overconsumption and contributes to weight gain (Figure 2) (13,15,34,37–39).

A diagram of obesity

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*Figure 2: Memory influences in a cyclical manner. Decreased hippocampal mnemonic functioning leads to decreased memory of meals and increased response to food cues. This leads to increased caloric consumption and obesity, which in turn leads to increased inflammation and cardiometabolic dysfunction which in turn decreases hippocampal function. {Figure extracted from Farr et al. (2016)}*

While existing evidence proposes that obesity can induce ID through chronic inflammation by increasing hepcidin production to disrupt iron absorption (3), our study introduces a crucial new dimension in the temporality of this association. By exploring how ID might act as a precursor to overweight and obesity through its effects on hippocampal volumes (Figure 3), we aim to illuminate a previously unrecognized pathway. Hippocampal volume serves as a proxy for structure, as reductions in volume are associated with structural alterations and cognitive impairment (40). Our approach addresses a significant gap in current knowledge and emphasizes the potential for early interventions targeting ID to prevent obesity risk, such as early targeted iron supplementation programs.

Adolescence represents an ideal stage for investigating the relation between obesity and ID due to the unique physiological and neurodevelopmental changes that occur during this period. Rapid growth during puberty significantly increases nutritional demands, particularly for iron (2,4,31). Research underscores that ID during adolescence can impair brain structure, including white matter integrity, and hinder cognitive function, with long-term consequences for learning and memory (6,7). Simultaneously, adolescents gain greater autonomy over their dietary choices, often leading to poor nutrition that exacerbates both ID and obesity risks (41–43). Adolescence thus provides a critical window for interventions aimed at addressing the intertwined challenges of ID and obesity.

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Figure 3: Conceptual framework for the relation between ID and risk of obesity

(*Variables in blue circles are directly measured in this study. Variables in pink represent hypothesized pathways that provide context but are not directly tested)*

***Innovation***:

The majority of studies on ID and obesity have focused on the mediating role of inflammation. Our study takes a different approach from existing research in a few key ways:

1. **Paradigm-Shifting Perspective**  
   We challenge the unidirectional view of the ID-obesity relation by proposing an innovative reverse-causation hypothesis. Traditionally, research has focused on how obesity disrupts iron metabolism through mechanisms like inflammation and increased hepcidin levels, which impair iron absorption and utilization. However, our hypothesis suggests that ID itself may contribute to the development or exacerbation of obesity. This study is the first to investigate hippocampal volume reductions as a potential mechanistic link between ID and obesity.
2. **Leveraging Big Data for Population-Level Insights**  
   By utilizing the NIH Adolescent Brain Cognitive Development (ABCD) study dataset, this research maximizes statistical power and generalizability. The integration of neuroimaging, iron biomarkers, and BMI trajectories provides a unique opportunity to study the complex relation between nutritional deficits, hippocampal structure, and obesity risk.
3. **Translational Potential to Public Health Interventions**  
   A finding that hippocampal volume is a mediator in the relation between ID and obesity, could pave the way for further investigation of the relation through animal studies and randomized controlled trials contributing to justification of the use of targeted iron supplementation strategies during childhood and adolescence to mitigate obesity risk.

***Research Approach****:*

This study leverages the ongoing and comprehensive longitudinal design of the ABCD dataset, which has been tracking nearly 12,000 children since 2017–2018, when participants were 9–10 years old, and will continue to follow them until they are 19-20 years old, allowing for the examination of developmental trajectories over time. The dataset includes repeated measures of brain structure and function (via MRI and fMRI), physical health, cognitive performance, and psychosocial outcomes, alongside detailed assessments of dietary habits, socioeconomic status, and various biospecimens. Most assessments are conducted annually, while imaging and biospecimen collection occurs once every two years. The dataset allows us to investigate the intersection between ID, hippocampal volumes, and risk of overweight and obesity during adolescence—a critical period for brain development and metabolic regulation. Our goal is to determine how variations in iron status are associated with hippocampal subregion/subfield volumes, and BMI-WC; and whether lower hippocampal volumes mediate the relation between ID and BMI-WC.

***Aim 1****:* ***Examine the associations between iron status and hippocampal subregion and subfield volumes in adolescents.***

***Rationale:*** Evidence from previous studies indicates that ID is associated with significant negative alterations in brain structure and function. Iron is essential for maintaining brain cytochrome c concentrations, a key component of the mitochondrial electron transport chain that drives ATP production (5). Reductions in iron levels can significantly impair the activity of cytochrome c oxidase, impairing cerebral energy metabolism and limiting the energy supply required for neuronal development and function. Hippocampal neurons, with their high energy demands, are particularly vulnerable making them susceptible to neuronal damage and changes in hippocampal morphology (4,17, 25). Iron deficiency-related structural and functional alterations, especially in the hippocampus and the frontal cortex, emphasize the importance of examining hippocampal subregion and subfield volumes in the context of iron deficiency.

***Data Preparation:*** We will extract T1-weighted (anatomical details) and T2-weighted (water content) structural MRI data processed using FreeSurfer’s longitudinal pipeline to ensure consistent segmentation of hippocampal subregions/subfields (the cornu ammonis regions (i.e. CA1, CA2, CA3, and CA4), dentate gyrus, and the subiculum) over time. We will extract data on iron biomarkers, that is serum ferritin and hemoglobin (Hb), at the first timepoint that these were measured in the dataset (T1) and consider follow-up year 4 (T2) and follow-up year 5 (T3) as our endlines for longitudinal analyses. Serum ferritin, while a key marker of iron storage, is also influenced by inflammation, which can lead to misinterpretation of iron status if not accounted for. Unfortunately, concentrations of specific markers of inflammation (such as C-reactive protein (CRP) or alpha-1-acid glycoprotein (AGP)) are not available in the ABCD dataset. Therefore, we will use white blood cell (WBC) count as a proxy for inflammation. WBC count is widely used in clinical practice as an indicator of inflammatory states and has been shown to correlate with systemic inflammation (45).

***Statistical Analysis:*** To investigate the relation between iron status markers and hippocampal subregion/subfield volumes, we will employ a multi-step analytical framework designed to capture both linear and non-linear associations, account for individual variability over time, and adjust for key confounders.

1. Serum ferritin levels will initially be modeled as a continuous variable using restricted cubic splines within regression models to explore potential non-linear relations with hippocampal subregion/subfield volumes. If non-linear trends are observed, we will retain this flexible modeling framework for subsequent analyses. If no significant non-linear patterns emerge, ferritin may be categorized into clinically meaningful groups (e.g., ID [ferritin <15 mg/mL] vs. iron sufficiency [ferritin =>15 mg/mL]). To account for repeated measures within participants over time, we will use linear mixed-effects models (LMMs) with random intercepts and slopes. These models allow us to capture both within-subject variability (e.g., changes in hippocampal volume over time) and between-subject differences (e.g., baseline ferritin levels). Fixed effects will include adjusted ferritin levels (accounting for inflammation using WBC counts), age, sex, SES, pubertal status, physical activity levels, stress levels, BMI-WC (to control for obesity-related hippocampal atrophy), and estimated total intracranial volume (eTIV). Prior to including these covariates in our models, we will conduct a collinearity assessment using Variance Inflation Factor (VIF) analysis to identify any highly correlated predictors. If VIF values exceed acceptable thresholds (e.g., >5 or >10), we will consider removing or combining correlated covariates to minimize collinearity concerns. Notably, while BMI-WC is included as a covariate due to its known impact on hippocampal volume, we will carefully evaluate its role to ensure it does not introduce collider bias in our analyses.
2. To disentangle the effects of ID from anemia-related impacts on appetite or brain structure/function, Hb will be included as a moderator in interaction terms within the LMM framework. This analysis will test whether the relation between ferritin levels and hippocampal volumes varies depending on Hb levels.
3. Sensitivity analyses will be conducted to evaluate the robustness of our findings by testing the impact of alternative assumptions, analytical approaches, and model specifications to ensure the stability and validity of our results.
4. Subgroup analyses will examine whether associations differ by sex or pubertal status.

***Expected results:*** We hypothesize that adolescents with ID (serum ferritin < 15 ng/mL) at T1 will show lower increases in hippocampal volumes between T1 and T2, while the iron-sufficient group is expected to demonstrate stable or increasing hippocampal volumes over the same period.

***Aim 2: Investigate the associations between iron deficiency and BMI-WC.***

***Rationale***: While obesity-induced inflammation is well-documented as a driver of ID through increased hepcidin production that disrupts iron absorption (46), our study explores a novel reverse pathway: whether ID contributes to adiposity through its effects on brain structure. Weight gain during adolescence is influenced by multiple factors including dietary intake, physical activity levels, genetic predisposition, and metabolic regulation. Emerging evidence suggests that disruptions in brain regions involved in appetite regulation—such as the hippocampus—may contribute to overeating behaviors associated with obesity risk.

Given the complex relationship between obesity and ID, this study will use Hb values as moderators. Anemia, and particularly iron deficiency anemia (IDA), is associated with decreased appetite and altered appetite regulation (47). Research has shown that individuals with IDA often have lower appetite scores. Furthermore, IDA can affect levels of ghrelin, a hormone that stimulates appetite (47). By including Hb values as moderators, this study aims to isolate the effects of ID on adiposity from the potential confounding effects of anemia-related changes in appetite and eating behavior.

***Data Preparation:***Anthropometric measures will be used to calculate BMI percentiles and categorized based on established standards for adolescents (underweight: <5th; healthy weight: 5th–<85th; overweight: 85th–<95th; obesity: ≥95th percentile) and waist circumference percentiles (>90th percentile for age/sex). Iron biomarkers include serum ferritin adjusted for inflammation using WBC count.

***Statistical Analysis:***To examine the relation between ID biomarkers and available adiposity measures:

1. We will model the potential non-linear relationship between adjusted serum ferritin levels and both BMI percentiles and waist circumference percentiles using restricted cubic splines. If no significant non-linear trends are observed for either measure, ferritin may be categorized into clinically meaningful groups.

2. LMMs will assess associations between adjusted ferritin levels and adiposity measures over time while accounting for repeated measures within participants. Given the potential correlation between BMI and waist circumference, we will consider modeling these two outcomes jointly or using composite measures to reduce Type I error rates associated with multiple dependent variables. Fixed effects will include Hb values as moderators or covariates to isolate anemia-related effects alongside other confounders such as age, sex, SES, sleep patterns, stress levels, physical activity levels. Composite measures will involve creating a single index that combines both BMI and waist circumference into one metric. This could be achieved through standardization (z-scores) of both variables followed by averaging or summing them to create a composite score that reflects overall adiposity. This approach allows us to capture the multifaceted nature of adiposity while addressing potential correlations between the two measures.

3. Sensitivity analyses will evaluate robustness across different adjustment strategies.

4. Subgroup analyses will assess whether associations differ by sex or pubertal status.

***Expected Results****:* We hypothesize that ID at T1 predicts higher BMI-WC at T2 and T3.

***Aim 3:******Test the mediating role of hippocampal subregion and subfield volumes in the associations between iron status and overweight/obesity.***

***Rationale:***  Previous studies have shown that ID is associated with alterations in hippocampal volume, a structural measure linked to cognitive and behavioral functions. The hippocampus plays a critical role in regulating eating behavior by integrating memory and decision-making processes. However, no study to date has systematically examined whether changes in hippocampal volume, potentially due to ID, mediate the relationship between ID and obesity-related outcomes.

***Data Preparation:***We will extract data on iron biomarkers (hemoglobin, ferritin) at T1, hippocampal subregion/subfield volumes (the cornu ammonis (CA) regions: CA1, CA2, CA3, and CA4, along with the dentate gyrus (DG), and the subiculum) at T2, and adiposity measures (e.g., BMI z-scores, waist circumference) at T2 and T3. This will ensure proper temporal ordering for longitudinal mediation analysis.

***Statistical Analysis:***To test mediation pathways:

1. Before conducting the full mediation analysis, we will first assess the direct relationships between hippocampal volume and both BMI and waist circumference (WC) separately. We will use linear regression models to evaluate these associations.
2. Conduct longitudinal mediation analysis using structural equation modeling (SEM). The analysis will include:

* Initially, we will test the total effect of ID on BMI-WC without considering the mediator.
* Next, we will assess the relationship between hippocampal volume and BMI-WC while controlling for ID. We will fit two types of mediation models:
  1. **Full Mediation Model**: This model will assess whether hippocampal volume mediates the relationship between ID and BMI-WC without a direct path from ID to BMI-WC.
  2. **Partial Mediation Model**: This model will include both paths—ID to hippocampal volume and hippocampal volume to BMI-WC—along with a direct path from ID to BMI-WC. We will compare model fit between these two models.

1. Include Hb moderation effects in SEM pathways to account for anemia-related effects on appetite regulation.
2. Use bootstrapping techniques to estimate confidence intervals for indirect effects.
3. Assess model fit using standard indices.

***Expected Results:***We hypothesize that lower hippocampal volumes mediate the relation between lower iron status and higher adiposity measures. This would reveal a potential mechanistic pathway linking nutritional deficiency with obesity risk during adolescence (Figure 4).



Figure 4: Directed acyclic graph (DAG) of the mediation analyses for the association between iron deficiency (ID) and obesity among adolescents in the ABCD Study mediated by hippocampal volume and controlling for confounding variables

The strength of our approach lies in its interdisciplinary collaboration, bringing together expertise across multiple fields to address this complex public health challenge. Our team includes specialists in iron deficiency and nutrient metabolism (Dr. Laura Murray-Kolb, PI of the project and dissertation mentor for the graduate student investigator), ingestive behavior and appetite regulation (Dr. Rick Mattes, dissertation committee member for the student investigator), childhood obesity and developmental trajectories (Dr. Kameron Moding, dissertation committee member for the student investigator), and secondary data analysis focusing on cardiometabolic disease (Dr. Qinglan Ding, co-investigator). By integrating these diverse perspectives, we are uniquely positioned to explore the connections between nutritional status, brain development, and eating behaviors, offering novel insights into the mechanisms linking ID to obesity risk.

**Potential Challenges and Mitigation Strategies:** We acknowledge potential challenges and will adopt strategies to promote scientific rigor and ethical integrity:

1. **Data Quality, Missing Values, and Reproducibility**: The ABCD dataset may contain missing or incomplete data, which can hinder the accuracy of our analyses. To mitigate this, we will employ robust techniques, such as multiple imputation, ensuring that our analyses are based on the most comprehensive dataset possible. Additionally, we will conduct sensitivity analyses to evaluate the potential impact of missing data on our findings, allowing us to assess the robustness of our conclusions. Upon completing our analyses, we will make our code available which will help with reproducibility of our findings.
2. **Lack of Correlations**: If no significant relations between ID, hippocampal volume reductions, and/or BMI-WC are found, we will explore proximal effects i.e. examine whether ID-related hippocampal volume reductions are associated with specific food choices (using dietary data) that could eventually lead to obesity. Null results will be reported to refine theoretical models and guide future research into alternative pathways connecting brain health and nutrition.

**References:**

1. Obesity and overweight [Internet]. [cited 2023 Jan 8]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

2. Aksu T, Ünal Ş. Iron Deficiency Anemia in Infancy, Childhood, and Adolescence. Turk Arch Pediatr 2023;58:358–62.

3. Alshwaiyat NM, Ahmad A, Wan Hassan WMR, Al-Jamal HAN. Association between obesity and iron deficiency (Review). Exp Ther Med 2021;22:1268.

4. Mesías M, Seiquer I, Navarro MP. Iron nutrition in adolescence. Crit Rev Food Sci Nutr 2013;53:1226–37.

5. Bastian TW, von Hohenberg WC, Mickelson DJ, Lanier LM, Georgieff MK. Iron Deficiency Impairs Developing Hippocampal Neuron Gene Expression, Energy Metabolism, and Dendrite Complexity. Dev Neurosci 2016;38:264–76.

6. Larsen B, Baller EB, Boucher AA, Calkins ME, Laney N, Moore TM, Roalf DR, Ruparel K, Gur RC, Gur RE, et al. Development of Iron Status Measures during Youth: Associations with Sex, Neighborhood Socioeconomic Status, Cognitive Performance, and Brain Structure. Am J Clin Nutr 2023;118:121–31.

7. Jahanshad N, Kohannim O, Hibar DP, Stein JL, McMahon KL, de Zubicaray GI, Medland SE, Montgomery GW, Whitfield JB, Martin NG, et al. Brain structure in healthy adults is related to serum transferrin and the H63D polymorphism in the HFE gene. Proc Natl Acad Sci Proceedings of the National Academy of Sciences; 2012;109:E851–9.

8. Georgieff MK. The Role of Iron in Neurodevelopment: Fetal Iron Deficiency and the Developing Hippocampus. Biochem Soc Trans 2008;36:1267–71.

9. Nelissen E, De Vry J, Antonides A, Paes D, Schepers M, van der Staay FJ, Prickaerts J, Vanmierlo T. Early-postnatal iron deficiency impacts plasticity in the dorsal and ventral hippocampus in piglets. Int J Dev Neurosci Off J Int Soc Dev Neurosci 2017;59:47–51.

10. Bastian TW, von Hohenberg WC, Georgieff MK, Lanier LM. Chronic Energy Depletion due to Iron Deficiency Impairs Dendritic Mitochondrial Motility during Hippocampal Neuron Development. J Neurosci 2019;39:802–13.

11. Lozoff B. Early iron deficiency has brain and behavior effects consistent with dopaminergic dysfunction. J Nutr 2011;141:740S-746S.

12. Carlson ES, Fretham SJB, Unger E, O’Connor M, Petryk A, Schallert T, Rao R, Tkac I, Georgieff MK. Hippocampus specific iron deficiency alters competition and cooperation between developing memory systems. J Neurodev Disord BioMed Central; 2010;2:133–43.

13. Kanoski SE, Grill HJ. Hippocampus Contributions to Food Intake Control: Mnemonic, Neuroanatomical, and Endocrine Mechanisms. Biol Psychiatry Elsevier BV; 2017;81:748–56.

14. Forloni G, Fisone G, Guaitani A, Ladinsky H, Consolo S. Role of the hippocampus in the sex-dependent regulation of eating behavior: Studies with kainic acid. Physiol Amp Behav Elsevier BV; 1986;38:321–6.

15. Davidson TL, Jarrard LE. A role for hippocampus in the utilization of hunger signals. Behav Neural Biol Elsevier BV; 1993;59:167–71.

16. Lister NB, Baur LA, Felix JF, Hill AJ, Marcus C, Reinehr T, Summerbell C, Wabitsch M. Child and adolescent obesity. Nat Rev Dis Primer Nature Publishing Group; 2023;9:1–19.

17. Kansra AR, Lakkunarajah S, Jay MS. Childhood and Adolescent Obesity: A Review. Front Pediatr 2021;8:581461.

18. Balasundaram P, Krishna S. Obesity Effects on Child Health. StatPearls. [Internet] Treasure Island (FL): StatPearls Publishing; 2024 [cited 2024 Dec 4]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK570613/

19. Anaemia [Internet]. [cited 2024 Dec 4]. Available from: https://www.who.int/health-topics/anaemia

20. Wang L, Liang D, Huangfu H, Shi X, Liu S, Zhong P, Luo Z, Ke C, Lai Y. Iron Deficiency: Global Trends and Projections from 1990 to 2050. Nutrients 2024;16:3434.

21. Miller JL. Iron Deficiency Anemia: A Common and Curable Disease. Cold Spring Harb Perspect Med 2013;3:a011866.

22. Dugdale M. Anemia. Obstet Gynecol Clin North Am 2001;28:363–81.

23. Al-Jermmy ASM, Idris SM, Coulibaly-Zerbo F, Nasreddine L, Al-Jawaldeh A. Prevalence and Correlates of Anemia among Adolescents Living in Hodeida, Yemen. Children 2022;9:977.

24. Gedfie S, Getawa S, Melku M. Prevalence and Associated Factors of Iron Deficiency and Iron Deficiency Anemia Among Under-5 Children: A Systematic Review and Meta-Analysis. Glob Pediatr Health 2022;9:2333794X221110860.

25. Bruner AB, Joffe A, Duggan AK, Casella JF, Brandt J. Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. The Lancet 1996;348:992–6.

26. Al-Naseem A, Sallam A, Choudhury S, Thachil J. Iron deficiency without anaemia: a diagnosis that matters. Clin Med 2021;21:107–13.

27. Weyand AC, Chaitoff A, Freed GL, Sholzberg M, Choi SW, McGann PT. Prevalence of Iron Deficiency and Iron-Deficiency Anemia in US Females Aged 12-21 Years, 2003-2020. JAMA 2023;329:2191–3.

28. Chang S, Wang L, Wang Y, Brouwer ID, Kok FJ, Lozoff B, Chen C. Iron-Deficiency Anemia in Infancy and Social Emotional Development in Preschool-Aged Chinese Children. Pediatrics 2011;127:e927–33.

29. Kim J, Wessling-Resnick M. Iron and mechanisms of emotional behavior. J Nutr Biochem 2014;25:1101–7.

30. Lozoff B, Corapci F, Burden MJ, Kaciroti N, Angulo-Barroso R, Sazawal S, Black M. Preschool-Aged Children with Iron Deficiency Anemia Show Altered Affect and Behavior,. J Nutr 2007;137:683–9.

31. More S, Shivkumar VB, Gangane N, Shende S. Effects of Iron Deficiency on Cognitive Function in School Going Adolescent Females in Rural Area of Central India. Anemia 2013;2013:819136.

32. Fretham SJB, Carlson ES, Georgieff MK. The Role of Iron in Learning and Memory12. Adv Nutr 2011;2:112–21.

33. Carlson ES, Tkac I, Magid R, O’Connor MB, Andrews NC, Schallert T, Gunshin H, Georgieff MK, Petryk A. Iron Is Essential for Neuron Development and Memory Function in Mouse Hippocampus. J Nutr 2009;139:672–9.

34. Parent MB, Darling JN, Henderson YO. Remembering to eat: hippocampal regulation of meal onset. Am J Physiol-Regul Integr Comp Physiol American Physiological Society; 2014;306:R701–13.

35. Rozin P, Dow S, Moscovitch M, Rajaram S. What Causes Humans to Begin and End a Meal? A Role for Memory for What Has Been Eaten, as Evidenced by a Study of Multiple Meal Eating in Amnesic Patients. Psychol Sci SAGE Publications Inc; 1998;9:392–6.

36. Kühnel A, Hagenberg J, Knauer-Arloth J, Ködel M, Czisch M, Sämann PG, Binder EB, Kroemer NB. Stress-induced brain responses are associated with BMI in women. Commun Biol Nature Publishing Group; 2023;6:1–15.

37. Stevenson RJ, Francis HM. The hippocampus and the regulation of human food intake. Psychol Bull American Psychological Association (APA); 2017;143:1011–32.

38. Togo J, Yang Y, Hu S, Liu J-J, Speakman JR. Effect of disrupted episodic memory on food consumption: no impact of neuronal loss of endophilin A1 on food intake and energy balance. J Genet Genomics Elsevier BV; 2022;49:329–37.

39. Farr OM, Li CR, Mantzoros CS. Central nervous system regulation of eating: Insights from human brain imaging. Metabolism Elsevier BV; 2016;65:699–713.

40. Doran S, Carey D, Knight S, Meaney JF, Kenny RA, De Looze C. Relationship between hippocampal subfield volumes and cognitive decline in healthy subjects. Front Aging Neurosci [Internet] Frontiers; 2023 [cited 2025 Feb 8];15. Available from: https://www.frontiersin.org/journals/aging-neuroscience/articles/10.3389/fnagi.2023.1284619/full

41. Ambrosini GL, Emmett PM, Northstone K, Howe LD, Tilling K, Jebb SA. Identification of a dietary pattern prospectively associated with increased adiposity during childhood and adolescence. Int J Obes 2005 2012;36:1299–305.

42. Ziegler AM, Kasprzak CM, Mansouri TH, Gregory AM, Barich RA, Hatzinger LA, Leone LA, Temple JL. An Ecological Perspective of Food Choice and Eating Autonomy Among Adolescents. Front Psychol 2021;12:654139.

43. Neufeld LM, Andrade EB, Ballonoff Suleiman A, Barker M, Beal T, Blum LS, Demmler KM, Dogra S, Hardy-Johnson P, Lahiri A, et al. Food choice in transition: adolescent autonomy, agency, and the food environment. Lancet Lond Engl 2022;399:185–97.

44. Ferreira A, Neves P, Gozzelino R. Multilevel Impacts of Iron in the Brain: The Cross Talk between Neurophysiological Mechanisms, Cognition, and Social Behavior. Pharmaceuticals 2019;12:126.

45. Chmielewski PP, Strzelec B. Elevated leukocyte count as a harbinger of systemic inflammation, disease progression, and poor prognosis: a review. Folia Morphol 2018;77:171–8.

46. Yanoff L, Menzie C, Denkinger B, Sebring N, McHugh T, Remaley A, Yanovski J. Inflammation and iron deficiency in the hypoferremia of obesity. Int J Obes 2005 2007;31:1412–9.

47. Ghrayeb H, Elias M, Nashashibi J, Youssef A, Manal M, Mahagna L, Refaat M, Schwartz N, Elias A. Appetite and ghrelin levels in iron deficiency anemia and the effect of parenteral iron therapy: A longitudinal study. PloS One 2020;15:e0234209.