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

**Background**

Obesity and iron deficiency (ID) are two widespread yet distinct public health crises, both with alarmingly high prevalence rates among adolescents worldwide. Obesity rates in adolescents have quadrupled since 1990 with over 390 million children and adolescents aged 5–19 years being overweight in 2022, including 160 million who were living with obesity (World Health Organization, 2024), and ID continues to remain the most common nutritional deficiency, affecting up to 21% of adolescents, especially females (Aksu & Ünal, 2023; Weyand et al., 2023). Emerging research suggests an intersection between these conditions (Alshwaiyat et al., 2021), however, the mechanisms connecting them are poorly understood. Drawing on recent nutritional and neuroscience discoveries, we propose an innovative mechanistic framework linking ID to obesity through hippocampal alterations.

**Hypothesis**

We hypothesize that ID leads to hippocampal atrophy, which disrupts appetite regulation, ultimately contributing to overeating and, consequently, overweight and obesity. This hypothesis emerges from two compelling sets of empirical data: (1) ID significantly impairs hippocampal development, affecting neuronal architecture and synaptic plasticity (Bastian et al., 2016; Nelissen et al., 2017), and (2) compromised hippocampal function directly disrupts appetite regulation by impairing meal-related memory and increasing responsiveness to food cues (Kanoski & Grill, 2017; Parent et al., 2014). Using the NIH comprehensive Adolescent Brain Cognitive Development (ABCD) dataset, we will systematically investigate if hippocampal deficits, as a consequence of ID, contribute to increased BMI and waist circumference:

***Specific aim 1: Examine the association between iron biomarkers and hippocampal subregion and subfield volumes in adolescents***  
Using serum iron biomarkers (hemoglobin, ferritin) and high-resolution structural MRI data, we will analyze how variations in iron status correlate with hippocampal subregion and subfield volumes.

***Specific aim 2: Investigate the association between iron status and adiposity, as measured by BMI and waist circumference***   
We will analyze the relationship between hippocampal subregion and subfield volumes and measures of adiposity, including BMI and waist circumference.

***Specific aim 3: Analyze if hippocampal atrophy is associated with adiposity, as measured by BMI and waist circumference***

Next, we will examine the association between serum iron biomarkers and measures of adiposity, that is, BMI and waist circumference

***Specific aim 4: Test the mediating role of hippocampal subregion and subfield volumes***  
 Using mediation analyses, we will evaluate whether hippocampal subregion and subfield volumes serve as a mechanistic link between iron deficiency and adiposity.

***Research Approach****:*

***Aim 1****:* ***Examine the association between iron biomarkers and hippocampal subregion and subfield volumes in adolescents***

***Rationale:*** Data from past studies strongly suggest that there are alterations in brain structure and function observed due to ID. Specifically, reductions in brain cytochrome c concentrations indicated altered cerebral energy metabolism, leading to inefficient ATP generation and electron transport. These alterations, particularly in the hippocampus and frontal cortex, set the stage for our investigation into hippocampal subregion and subfield volumes and their relationship with iron status (3).

***Data Selection:***Extract T1-weighted and T2-weighted structural MRI data processed using FreeSurfer’s longitudinal pipeline for segmentation of hippocampal subregions (e.g., anterior/posterior hippocampus) and subfields (e.g., CA1, CA3, dentate gyrus). Also obtain measures of anemia and iron biomarkers data (hemoglobin, ferritin). Ensure data is harmonized across different scanners and acquisition protocols.

***Statistical Analysis:***

1. Classify participants into ID and iron-sufficient groups using clinical cutoffs (Hb < 12 g/dL, Ft < 15 mg/mL). Compare hippocampal subregion and subfield volumes between these groups using:
   * Independent t-tests or Mann-Whitney U tests for cross-sectional comparisons.
   * LMMs to assess group differences over time while adjusting for covariates.
2. Conduct multivariate regression to examine the association between serum ferritin levels and hippocampal volumes. Adjust for confounders, including age, sex, socioeconomic status (SES), pubertal status, physical activity levels, estimated total intracranial volume (eTIV), etc
3. Use LMMs to assess the relationship between iron biomarkers at baseline and hippocampal volumes over time.
4. Include random intercepts and slopes to account for individual variability in baseline levels and rates of change.

***Expected results:*** We hypothesize that adolescents with iron deficiency (serum ferritin < 15 mg/mL) will exhibit a greater degree of hippocampal atrophy compared to iron-sufficient participants, after controlling for confounders.

***Aim 2: Investigate the association between iron status and adiposity, as measured by BMI and waist circumference in adolescents***

***Rationale*** While obesity-induced inflammation is well-documented as a driver of ID through increased hepcidin production that disrupts iron absorption (14), our study explores a novel reverse pathway: whether ID contributes to adiposity through its effects on brain structure and function.

***Data Preparation:***

Extract measures of anemia and iron biomarkers (hemoglobin, ferritin) alongside anthropometric measures of adiposity (BMI z-scores and waist circumference).

***Statistical Analysis:***

* Use LMMs to examine associations between iron biomarkers and adiposity measures while adjusting for confounders such as SES, physical activity levels, eTIV, pubertal status, sex, scanner type, etc.
* Explore potential non-linear relationships using GAMMs, if necessary.

***Expected Results****:* We hypothesize that lower iron status will be associated with higher BMI z-scores and waist circumference in adolescents.

***Aim 3: Analyze if hippocampal atrophy is associated with adiposity, as measured by BMI and waist circumference***

***Rationale:*** The hippocampus plays a crucial role in regulating eating behavior through memory-dependent mechanisms and integration of metabolic signals. Animal studies demonstrate that hippocampal dysfunction leads to increased meal frequency and altered satiety signaling. The vHP particularly integrates metabolic hormone signals with environmental food cues (15).

***Data Selection:*** Extract measures of BMI z-scores and waist circumference alongside quality-controlled hippocampal subregion/subfield volumes from structural MRI data. Ensure data is harmonized across different scanners and acquisition protocols.

***Statistical Analysis:***

* Implement LMMs with lagged predictors to assess whether hippocampal volumes predict BMI z-scores and waist circumference while controlling for baseline levels.
* Include covariates such as eTIV, SES, pubertal status, sex, scanner type, etc.
* Test interaction effects between specific hippocampal subregions (e.g. left vs right, vHP vs dHP) and adiposity measures to identify region-specific contributions.
* Examine potential non-linear relationships using GAMMs, if necessary.
* Assess longitudinal trajectories of hippocampal atrophy and adiposity to identify critical developmental windows of vulnerability.

***Expected Results****:* We hypothesize that low hippocampal volumes will be associated with higher BMI z-scores and waist circumference.

***Aim 4:******Test the mediating role of hippocampal subregion and subfield volumes***

***Rationale:*** The relationship between iron status and adiposity may be mediated by changes in the hippocampal structure. While previous studies have independently linked iron status with hippocampal structure and hippocampal function with eating behavior, no study has systematically tested whether hippocampal volume mediates the relationship between ID and obesity-related outcomes.

***Data Preparation:***

Extract data on iron and anemia biomarkers (hemoglobin, ferritin), anthropometric measurements (BMI z-scores, waist circumference), and hippocampal subregion/subfield volumes.

***Statistical Analysis:***

* Conduct longitudinal mediation analysis using structural equation modeling (SEM) to test whether hippocampal volume mediates the association between iron biomarkers and adiposity measures.
* Use bootstrapping techniques to estimate confidence intervals for indirect effects while assessing model fit using standard indices.
* Test moderated mediation effects by covariates such as sex or SES if applicable.

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