Maternal Choline Supplementation and Offspring Cognition in Adolescence

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This study seeks to test the general hypothesis that maternal choline intake of 930 mg/d throughout the 3rd trimester of pregnancy, compared to 480 mg/d, causes offspring to have superior cognitive functioning. Three domains of cognition will be examined to test three independent hypotheses about the effects of the intervention on (1) sustained attention, (2) executive functioning, and (3) memory.

Sample Size:

The sample size was determined by the original controlled feeding study, which was designed to detect group differences for that study's primary endpoints—biomarkers of choline status—for which it was estimated that a sample size of 26 (13 per treatment group) would achieve 80% power to detect a moderate effect size at an α of 0.05. The sample sizes for the ancillary longitudinal follow-up studies to investigate treatment effects on infant and child cognitive outcomes was limited by the original sample size, minus loss to follow-up. The results of these studies demonstrated that (1) a sample size of 24 (12 per group) was sufficient to detect a statistically significant treatment effect of the prenatal choline intervention on infant processing speed (Caudill, et al., 2018) and (2) that a sample size of 20 (11/9 participants from the 930/480 mg choline/d groups, respectively) was sufficient to demonstrate group differences on several cognitive outcomes age 7 years. No further sample size calculations were conducted for this 14-year follow-up study because the sample was limited to those included in the original feeding study, which was sufficient to see the effect of choline on cognition. We hypothesize that the cognitive effects of the prenatal supplementation should endure into adolescence, and we expect that the cognitive outcomes will be measured with much greater precision at age 14 years that during infancy or at 7 years, thus we expect to have adequate power to detect similarly moderate to large effect sizes.

Descriptive Statistics:

Univariate and bivariate descriptive statistics and graphs will be examined to assess data value plausibility. Descriptive statistics for sample characteristics, by group, will be presented in tables. Continuous variables will be summarized with means and standard deviations, and categorical variables will be summarized with absolute counts and relative frequencies. A table of sample characteristics will include participant sex, level of education, parental level of education, native language, family income, history of head injury absence/presence, and concurrent medications and supplements. We will also provide a flow chart detailing participant attrition and missing data.

Inferential Statistical Analysis:

Acknowledging the risks inherent with utilizing multiple endpoints in a small sample, we generated an *a priori* analysis plan, with specific objectives in alignment with the areas of cognition of interest. For each cognitive objective, we have detailed the tasks and outcome variables that will be used for its measurement.

Objective 1 Sustained Attention: Estimating the effect of maternal third trimester choline intake on offspring **sustained attention** at age 14 years.

- **Sustained Attention** will be measured using the Rapid Visual Information Processing task (RVP) of the Cambridge Neuropsychological Test Automated Battery (CANTAB).
    - **The primary outcome for this objective will be the nonparametric index of detection sensitivity, A-prime.**
        - A-prime will be analyzed using a general linear model with fixed effects of treatment group and participant sex.

Objective 2 Executive Functioning: Estimating the effect of maternal third trimester choline intake on offspring **executive functioning** at age 14 years.

- Our primary outcome for this objective will be a composite measure of executive functioning (EF) derived from key variables obtained from multiple tasks that assess four core components of EF: planning, working memory, inhibitory control, and attentional flexibility. All tasks are administered using the CANTAB platform.
    - **Planning** will be assessed by two versions of a Towers of London task. The key outcome variable from the **Stockings of Cambridge task (SOC) will be the number of perfect solutions across all problems.** From the **One Touch Stockings of Cambridge task (OTS) the key outcome** variable will be the number of problems solved on the first attempt.
    - **Working Memory** will be assessed using the **Spatial Working Memory task (SWM)** and the Backward Digit Span task (BDS). The key outcome variable for SWM will be the total number of search errors and span length for BDS.
    - **Inhibitory control** will be assessed using **the Cambridge Gambling Task (CGT).** The key outcome variable will be the delay aversion total score.
    - **Attentional flexibility** will be assessed using the **Intradimensional-Extradimensional Shift task (IED).** The key outcome variables will be the total number of choice errors.
- The EF composite (EF_{comp}) outcome variable will be defined as the average z-score of the individual key EF task outcomes as follows: EF_{comp} = (zSOC perfect solutions+ zOTS first attempt correct+ $_z$SWM search errors+ $_z$BDS span length+ $_z$CGT delay aversion+ $_z$IED choice errors) / 6

with the z-score calculated by the sample mean and standard deviation:

$$z = \frac{raw\ score - \bar{x}_{\square}}{sd_{\square}}$$

Statistical analysis of EF_{comp} will be conducted using a general linear model with fixed effects of treatment group and participant sex.

Objective 3 Memory: Estimate the effect of maternal third trimester choline intake on offspring **memory function** at age 14 years.

- Our primary outcome for this objective will be a composite measure of memory function derived from multiple tasks assessing episodic memory, recognition memory, and working memory. All tasks are administered using the CANTAB platform.
- **Episodic memory** will be assessed by a Verbal Paired Associate Learning task (VPA) and a nonverbal **Paired Associate Learning task (PAL).** The key outcome for both tasks will be the total number of errors.
- **Recognition memory** will be assessed by the **Delayed Match to Sample task (DMS) and the Pattern Recognition Memory task (PRM).** The key outcome for both tasks will be the proportion of correct choices on delay trials.
- **Working memory** will be assessed using two span tasks: **nonverbal spatial span (SSP**) and verbal forward digit span (FDS). The key outcome variable for both will be span length.

- The memory composite (MEM_{comp}) outcome variable will be defined as the average z-score of the individual key outcomes from the memory tasks as follows: MEM_{comp} = (zVPA total errors+ zPAL total errors+ $_z$DMS proportion correct+ $_z$PRM proportion correct+ $_z$SSP span length+ $_z$FDS span length) / 6

  with the z-score calculated by the sample mean and standard deviation:

$$z = \frac{raw\ score - \bar{x}}{sd}$$

  Statistical analysis of MEM_{comp} will be conducted using a general linear model with fixed effects of treatment group and participant sex.

Exploratory Objective: Psychological Health

- We will use the ASEBA-web Youth-Self Report (YSR) scores that align with the DSM-V. We will compare depressive problems scores, anxiety scores and attention scores from the two groups.

Missing Data

We will use descriptive statistics (n for each measure) to quantify and report the extent of any missing data. Reasons for missing data will be clearly documented, categorized, and reported in publications. We will use the multiple imputation technique, which involves generation of multiple data sets with imputed values, which are then pooled. From this pooled imputed data set, we will conduct sensitivity analyses to assess the effect of the imputations on the results and compare the findings with and without the imputations to verify the strength of our results.

Secondary, post-hoc, and exploratory analyses

Given our small sample size, the complexity of the interpretation of cognitive tests, and the novelty of this research. We will, as appropriate conduct additional planned secondary and

sensitivity analyses, in addition to post-hoc exploratory analyses to better understand our data to arrive at the fullest possible understanding of the effects of the intervention.