University of Kansas Medical Center
RESEARCH PROTOCOL INVOLVING HUMAN SUBJECTS
TEMPLATE WITH GUIDANCE

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Study Title: Added sugar intake and brain structure and function

Co- Investigator(s): Laura Martin, PhD

I. Purpose, Background and Rationale

A. Aim and Hypotheses

1. The first 1000 days post-conception have been characterized as a critical window for brain development, during which much of the brain's growth, structure, and function is set[1]. Key nutrients from maternal and infant dietary intake fuels these maturational processes, thus providing the potential underlying foundation for later cognitive development. Brain growth and development is particularly sensitive to poor nutrition during this window[2]; previous work detailed the effects of deficiencies in the status of folic acid, choline, iron, zinc, iodine, and long chain polyunsaturated fatty acids[3]. While nutrient deficiencies have been studied, less is known about the impact and timing of excess nutrition in relation to cognitive development[4].

2. Aim 1: Determine the relationship between added sugars (% calories) intake during pregnancy and infancy/toddlerhood (birth to 24 months) on brain structure and function. Hypothesis: added sugars intake will be negatively related to brain structure (hippocampal volume) and function (resting state functional connectivity and P3 amplitude). We will explore how sex modifies this relationship.

   Aim 2: Determine the relationship between fructose (% calories) consumption during pregnancy and infancy/toddlerhood (birth to 24 months) on brain structure and function. Since some studies reported relationships with fructose independently, we will explore relationships with fructose separately.
   Hypothesis: fructose intake will be negatively related to brain structure (hippocampal volume) and function (resting state functional connectivity and P3 amplitude). We will explore how sex modifies this relationship.

B. Background and Significance

1. Study Significance: Brain development is a protracted process that begins in early gestation and continues into late adolescence. By the 8th week in gestation, the basic brain structures and the central nervous system are formed, and neuron production and migration are started[5]. From the 8th week until delivery, the cortical and subcortical structures form, including the major fiber pathways. In the third trimester, myelination

and growth of the hippocampus increases rapidly until around18-24 months of age[1]. Rapid brain growth continues with a four-fold increase in brain size during early childhood, reaching 90% of full brain size by six years old. The first 1000 days post-conception have been characterized as a critical window for brain development, during which much of the brain's growth, structure, and function is set[1]. During this time, the brain is particularly vulnerable to nutrient deficiencies, and such deficiencies can have a severe impact on cognitive development[2,3]. Promoting optimal development and cognition has clear individual benefits, but also has substantial economic impact[6-8]. The prevention of later developmental deficits is estimated to save $6B annually[9,10].

The concept that early development disproportionately affects later outcomes is widely accepted in the developmental sciences[11-15], and the nutrition literature[16-22]. Thus far, most research has focused on key early life nutrient deficiencies[2,4,16]. Prenatal and postnatal long-chain polyunsaturated fatty acids (LCPUFA)[23] docosahexaenoic acid (DHA) and arachidonic acid (AA), iron[24-27], and zinc[28-30] promote brain structure development, myelination, neurogenesis, neuronal migration, synaptogenesis and production of neurotransmitters. Iron deficiency is related to decreased language skills and fine-motor skills[31,32] and supplementing iron, copper, and zinc improved attention measures[33]. Nutrient intake strongly supports the normal development of underlying neural structure and function that is critical for later cognitive development. While the effect of nutrient deficiencies is reported, the impact of excess nutrition is less studied.

2. The impact of excess nutrient intake on brain development and function has garnered far less consideration compared to nutrient deficiencies. Data suggest that added sugars intake during pregnancy, infancy, and toddlerhood may interfere with the early formation of the brain structures that contribute to learning and memory[34]. Fructose is of particular interest here, as it is one of six monosaccharides represented in added sugars[35], and is found in the diet as a sweetener commonly added to foods during processing in the form of high fructose corn syrup (HFCS) and sucrose, a disaccharide made of a glucose bound to a fructose[36]. HFCS is a low-cost sweetener commonly used in sugar sweetened beverages (SSB). The effect of fructose may be due to fructose metabolism not being tightly regulated like glucose metabolism[36] leading to oxidative stress and an inflammatory response[37].

3. Literature Review: The literature suggests that added sugars intake in early life exceeds recommendations. Added sugars are defined as sugars and syrups added as an ingredient to foods during processing, preparation, or at the table, while total sugars include added sugars plus sugar naturally occurring in foods (e.g., fruit and milk)[35]. One added sugar in particular, fructose (e.g., high fructose corn syrup), has been investigated as a driver of the negative relationships reported between added sugars and health outcomes[38]. Pregnant women are recommended to consume <10% of calories/day from added sugars, whereas infants and toddlers (<24 months) are recommended to consume no added sugars[39]. Even so, pregnant women are reported to consume 15.8% of calories/day from added sugars[40], and 6- to 24-month-olds to consume 1.4-7.2% of calories/day from added sugars[41,42]. Data in pregnant women and infants and toddlers

found 70% of pregnant women[39] and 97.3% of infants and toddlers consumed added sugars in each day[43]. While this is concerning given the relationships between added sugars intake and chronic disease development[44-46], evidence is growing that early added sugars intake is also related to diminished cognitive function[47].

In adolescent rodents and humans, added sugars (including fructose) negatively impacted the hippocampal structure and connectivity[48], increased hippocampal inflammation[49], and impaired hippocampal-dependent memory[50]. Human data from the prenatal period and infancy are lacking, but one published study[34] found greater maternal prenatal consumption of added sugars was related to poorer cortical organization, and greater maternal fructose intake during lactation was related to lower scores in the offspring on the Bayley-III Scales of Infant Development at 24 months old[51]. These preliminary studies are consistent with the hypothesis that added sugars intake during pregnancy, infancy, and toddlerhood may interfere with the development and early formation of neural circuits[34], perhaps owing to increased oxidative stress/inflammation[49,52,53] or decreased production of brain derived neurotrophic factor (supports brain development and circuit formation)[52,54], leading to suboptimal cognitive outcome. This hypothesis was further supported by preliminary data from our own laboratory, which found that added sugars intake during the first two years of life was related to lower scores on standardized cognitive tests from 18 months to 6 years of age. The purpose of this proposal is to understand the relationship between maternal and early offspring (birth to 24 months) added sugars intake and brain structure and function at 5-6 years of age.

In 7–11-year-old children[48], increased intake of fructose and glucose was associated with increased right hippocampal CA2/3 subfield volume, and increased fructose intake was associated with mean diffusivity in the right cingulate-prefrontal cortex connections. This suggests an impact on hippocampal structure related to an increase in hippocampal inflammation. In the Project Viva cohort[55], greater maternal prenatal intake of SSB was related to poorer offspring global intelligence. Greater maternal prenatal added sugars consumption was also related to lower newborn gray matter at 3 weeks old, including areas for later cognitive functioning[34], suggesting an impact on formation of dendrites and synapses. This cohort was tested again at 24 months using the Bayley-III Scales of Infant Development[56]. Greater maternal SSB intake was related to lower scores on the Bayley-III. These data provide evidence for a relationship between maternal added sugars intake and the brain structures that are the foundation for future cognitive development.

C. Rationale

1. As described above, there is a growing body of evidence to suggest that excess added sugar intake negatively affects cognition.
2. The innovation for this proposal involves a shift with respect to the theoretical approach around the relationship between cognition and nutrition. *While the impact of key nutrient deficiencies on cognition is well documented, the relationship between excess intake (i.e., added sugars) and cognitive development has not been previously studied.* Further, data are lacking on exposure timing relative to the impact on cognition and brain

development. This proposal will investigate three time periods that are well established windows of brain development that are sensitive to nutrient exposures: the prenatal, infancy, and toddlerhood time periods.

3. n/a – this study is not designed to improve the diagnosis or treatment of a disease

II. Research Plan and Design

A. Study Objectives: This purpose of this study is to fill a gap in the literature by providing an understanding of the relationship between added sugars and fructose intake during sensitive windows to brain structure and function. This project is unique because we can determine the effect of added sugars during three different times: pregnancy, infancy, and toddlerhood.

Primary Aims:
Aim 1: Determine the relationship between added sugars (% calories) intake during pregnancy and infancy/toddlerhood (birth to 24 months) on brain structure and function.

Aim 2: Determine the relationship between fructose (% calories) consumption during pregnancy and infancy/toddlerhood (birth to 24 months) on brain structure and function.

Secondary Aims:
Secondary Aim 1: Determine the relationship between added sugars (% calories) intake during pregnancy and infancy/toddlerhood (birth to 24 months) on cognition.

B. Study Type and Design: This is a non-interventional, observational study.

Added sugar intake during pregnancy will be assessed using dietary data collected between 12-20 wks gestation using the using the National Cancer institute (NCI) DHQ-II food frequency questionnaire (FFQ) or three 24-hour dietary recalls.

Offspring dietary intake was collected at 2 weeks and at 6, 12, and 24 months by one multiple-pass 24-hour dietary recall administered to the child's caregiver and collected by trained research staff.

For the present study, we will collect three total dietary recalls: two weekday and one on the weekend.

Brain structure and function will be measured using MRI and EEG assessments. Cognition will be measured using dimensional change card sort task and Peabody picture vocabulary test.

C. Sample size, statistical methods, and power calculation

1. n/a – randomization will not be used
2. n/a – study will not be blinded
3. The present study is pilot. The planned sample size is 45 subjects.

D. Subject Criteria (See Vulnerable Populations appendix, if applicable): Participants who were previously enrolled in the ADORE GAINS study will be invited to participate in the present study when they are 5-6 years old. Participants will be both male and female and English or Spanish speaking.

1. Inclusion criteria: Participated in ADORE GAINS. Age 5-6.
2. Exclusion criteria: children who have received an autism diagnosis (per parent report)
3. Withdrawal/Termination criteria: n/a

4. Subjects may enter another research study while participating in this research study, as this study is observational only.

E. Specific methods and techniques used throughout the study

1. Laboratory tests: n/a
2. Study Procedures:
   Dietary assessment: Three multiple-pass 24-hour dietary recall will be administered to the child/child's caregiver and collected by trained research staff to characterize energy and nutrient intake of the child. 24-hour recalls accurately estimate dietary intake[57,58] and contain less reporting bias than diet records[57,59]. The recalls are entered into the Nutrition Data System for Research (NDS-R, version 2021, Minneapolis, MN) for macro- and micronutrient analysis including added sugars.

   Dimensional Change Card Sort (DCCS) task. This test is based on the basic paradigms of discrimination learning[65,66], which was a focus of our laboratory's research program during the 1990s[67,68]. This particular task is administered according to the protocol published by[69], and has also been used in other nutritional studies, as well as in our research program on the development of attention in preschoolers[70]. Essentially, participants are presented with stimuli that can be sorted on various dimensions (e.g., shape or color). They are asked to sort the stimuli on one dimension (thus providing a measure of performance on that ability) but then are asked to reverse (i.e., "switch") the rule. At that point, they must inhibit the previously activated rule in order to perform correctly under the new rules. For this particular task, children are presented a game in which they must sort 7 "red bunny" cards and 7 "blue car" cards into one of two boxes. One box is "labeled" with a red car and the other with a blue bunny. The child is asked to play either the "color game," with red bunnies sorted (face down) into the "red car" box; blue cars sorted (face down) into the "blue bunny" box; or they are asked to play the "shape game," with blue cars sorted (face down) into the "red car" box and red bunnies sorted (face down) into the "blue bunny" box. The protocol is to administer two practice trials, and then 6 trials with varying levels of support as needed (i.e., instructions may be repeated, or cards may be identified as a scaffold for the production of strategies) although the answers are never given to the child. If children perform 5 out of 6 trials correctly, the game is switched to the opposite (i.e., shape-to-color or color-to-shape). Should the child successfully negotiates the switch, the task would proceed with the "border version" of the task, where half of 12 sorting cards have a black border around the picture, and the child is asked to play "color game" when the card has a black border and "shape game" when it does not; here, there are 12 trials, but no game-switch phase. Correct responses are recorded for each phase, and performance is also qualitative characterized as 0 (fail pre-switch phase), 1 (pass pre-switch, fail post-switch), 2 (pass the pre- and post-switch phases, but fail border phase), and 3 (pass all phases). The DCCS task will take approximately 15 minutes.

   Peabody Picture Vocabulary Test (PPVT). Receptive vocabulary will be assessed using the PPVT-4. The PPVT-4 is a nationally standardized assessment of receptive vocabulary that has been shown to be fair with respect to cultural and economic status[71]. In this task,

participants are shown pages depicting 4 pictorial choices. For each page, the examiner says a word, and the participant indicates, either by pointing or by stating a reference number, which picture best matches the word. Administration is simple and straightforward. The PPVT-4 yields a standard score normed with a mean of 100 and a standard deviation of fifteen. It takes about 30 minutes to complete.

If one or both cognitive tests (DCCS and PPVT) are not completed at the second study visit for any reason, such as subject fatigue, the study team can ask the family if they are willing to come back at a later date for completion of the test(s). Compensation of an additional $50 would be provided if an additional visit is completed.

Dual energy x-ray absorptiometry (DXA) scan: (Prodigy, Madison, WI, encore software version 13.60) will be used to measure body composition and regional AT distribution. The DXA is located within the Dietetics & Nutrition Clinic inside the Smith West building at KUMC. Using specific anatomic landmarks as previously described, regions including the arms, legs and trunk will be demarcated[72]. Calculations for FM in each region and summed for regions comprising the central (trunk) and peripheral (arms plus legs) will be completed. If a child moves during the scan the scan may need to be re-done but no more than 2 scans will be given over the duration of the study. The DXA scan takes about 10 minutes.

If there is movement during the first DXA scan and the study team and/or parent does not think the child will lay still for a second scan, the family may be asked if they would come back a different day to complete a second DXA. As stated above, no more than 2 scans will be given over the duration of the study. Compensation of an additional $50 would be provided if an additional visit to repeat the DXA scan is completed.

Anthropometry: Body weight will be assessed on a same calibrated scale (Detetco Scales, Webb City, MO). Standing height will be measured using a wall mounted stadiometer (Accu-Hite, Seca Corp, Hanover, MD). Subjects will remove shoes and be centered on the stadiometer. Height will be recorded to the nearest 0.1 cm. Two measurements will be taken and the average will be recorded. The measurements take about 5 minutes to complete.

Skinfolds: Six skinfolds will be measured to represent AT distribution in order to compare to larger trials that do not have DXA. Dr. Hull has published using these methods to assess infant FM distribution[73] and it has been argued skinfolds are a valid metric of regional FM[74]. All measurements will be collected using standardized procedures to our Laboratory and will take place on the same day as the body composition assessment by DXA using standardized procedures. All skinfolds will be identified using anatomical landmarks and taken on the right side of the body using Lange calipers (Beta Technology, Santa Cruz, CA). Skinfolds will be taken in order from head to toe and then repeated in that same order. If two skinfold measurements differ by more than 1 mm, a third measurement will be taken. The two measurements within 1 mm will be averaged and used for analysis. Biceps and triceps skinfolds will be measured at the midline of the

anterior and posterior surface of the arm, respectively, on the mid-point between acromial process of the scapula and olecranon process of the ulna. Subscapular skinfold will be measured at the lower angle of the scapula. Suprailiac skinfold will be measured anteriorly to the midaxillary line and superiorly to the iliac crest, along the natural cleavage of the skin. The thigh skinfold will be measured at the mid-point between patella (kneecap) and inguinal crease at the anterior surface of the thigh. Flank skinfold will be measured immediately above the iliac crest at the mid-axillary line. Central FM will be calculated by adding the subscapular, suprailiac and flank skinfolds and dividing by two. Peripheral FM will be calculated by adding the thigh, biceps and triceps skinfolds and dividing by three. The skinfolds take about 15 minutes to complete.

If the anthropometric and/or skinfold measurements are not completed at the second study visit, for any reason, the study team can ask the family if they are willing to come back at a later date for completion of the measurements. Compensation of an additional $50 would be provided if an additional visit is completed.

3. Measuring height and weight are part of usual care for this population. No other procedures are part of usual care. None of the tests or measurements will be billed to insurance.
4. n/a – no tissue samples will be collected
5. Timeline:

Procedure	**Study Visit 2**
Questionnaires	x
Diet Recall (3 total)	x
MRI acclimation scan	
MRI scan	
EEG	
Card sorting game	x
Picture vocabulary test	x
Anthropometry & skinfolds	x
DXA scan	x

F. Risk/benefit assessment: No appreciable risk of physical, psychological, social, legal or other harm is expected.

Physical risk: The DXA exposes the subject to a small amount of radiation during the scan. One DXA scan is equivalent to approximately 2 hours of natural background radiation. To minimize radiation exposure, no more than two DXA scans will be completed for this study. Subjects may feel a light pinch when calipers are used to measure skinfolds. Study staff will be adequately trained and use gentle techniques to minimize discomfort.

1. Psychological risk: Some children may get tired during the cognition tests and not perform at their best
2. Social risk: n/a
3. Economic risk: n/a

4. Potential benefit of participating in the study
    a. n/a – no direct benefit to the individual subject or parent
    b. n/a – no direct benefit to the population from which the subject is drawn
    c. The information gained in this study will fill a gap in the literature by providing an understanding of the relationship between added sugar intake during a sensitive window of brain development. This data will help inform public health recommendations for added sugar intake and inform when interventions should occur.

G. Location where study will be performed: All study visits will occur at the KU Medical Center main campus in Kansas City, KS. The study visit will occur at Dr. Hull's research laboratory in the SmithWest building. All participants will be assigned a non-identifiable subject number. Subjects' records will be stored electronically on the KUMC RedCap server. All paper records will be kept in Dr. Hull's locked research laboratory. Only approved study team members have access to this area.

H. Collaboration (with another institution, if applicable): n/a

I. Single IRB Review for a Multi-site study (if applicable): n/a – not a multi-site study

1. For which sites will KUMC serve as the IRB of record? n/a
2. Indicate which study activities will occur at each site. If all study procedures will be identical across study sites, state this. n/a
3. Describe how you will assess the capacity of each site to perform the research (e.g., expertise, staffing, space, equipment, etc.) If applicable, include site evaluation tools in your IRB submission. n/a
4. Describe how the lead investigators will ensure that all participating sites use the IRB-approved version of the protocol, consent, recruitment materials and other study documents. n/a
5. Describe how the lead investigators will communicate with and disseminate new information to other sites (e.g., training meetings, regularly-scheduled conference calls, notifications, etc.) n/a
6. Describe how the lead investigator will assess protocol compliance, unanticipated problems and adverse events at other sites. n/a
7. Name the member of the KUMC study team who will be the point of contact to coordinate oversight and communication with the sites. n/a

J. Community-Based Participatory Research (if applicable)

1. Participants and the nature of their involvement: n/a
2. Cultural issues: n/a
3. Origin of the research question: n/a
4. Risks and Benefits: n/a
5. Study Description and Process: n/a
6. Return of results: n/a
7. Sustainability: n/a

K. Personnel who will conduct the study, including:

1. Indicate, by title, who will be present during study procedure(s):
    a. Sarah Crawford: Study Coordinator, all visit procedures
    b. Laura Castro de Santiago: Bilingual Study Coordinator, all visit procedures
    c. Jill Shaddy-Gouvion: Research Personnel, will perform cognitive testing
2. Primary responsibility for the following activities, for example:
    a. Determining eligibility: Sarah Crawford, Laura Castro de Santiago
    b. Obtaining informed consent: Sarah Crawford, Laura Castro de Santiago,
    c. Providing on-going information to the study sponsor and the IRB: Sarah Crawford, Laura Castro de Santiago
    d. Maintaining participant's research records: Sarah Crawford, Laura Castro de Santiago
    e. Completing physical examination: n/a
    f. Taking vital signs, height, weight: Sarah Crawford, Laura Castro de Santiago (height and weight only)
    g. Drawing / collecting laboratory specimens: n/a
    h. Performing / conducting tests, procedures, interventions, questionnaires: Sarah Crawford, Laura Castro de Santiago, Jill Shaddy-Gouvion
    i. Completing study data forms: Sarah Crawford, Laura Castro de Santiago
    j. Managing study database: Sarah Crawford, Laura Castro de Santiago

L. Assessment of Subject Safety and Development of a Data and Safety Monitoring Plan

1. Elements of the plan include:
    a. Persons/groups who will review the data (study team; independent safety monitor, data monitoring committee or formal DSMB): PI and study team
    b. Data/events that will be reviewed: n/a
    c. Frequency of review: n/a
    d. Types of analyses to be performed: n/a
    e. Safety-related triggers that would cause the PI to stop or alter the study: n/a
2. n/a – non-interventional study, no anticipated adverse events related to the study
3. n/a – non-interventional study, no anticipated adverse events related to the study

III. Subject Participation

A. Recruitment:

1. Subjects will be recruited from the ADORE GAINS study cohort if they agreed to future contact on the informed consent form at the time of consent for that study.
2. Study coordinators and graduate research assistants will contact potential participants by phone. The study will be explained using the recruitment script (pending IRB approval).
3. n/a – no advertisements or flyers will be used for recruitment

4. The recruitment diagram and recruitment postcard will be sent to potential participants who express interest when spoken to on the phone and agree to have the materials sent electronically. These handouts are intended to provide a simple, visual overview of what the entails. They will be accompanied by the more detailed verbal explanation provided by the study team via phone.

B. Screening Interview/questionnaire: The general health screener will be sent to parent to complete. This has questions to assess MRI safety and if the child has been diagnosed with autism.

C. Informed consent process and timing of obtaining of consent

1. Sarah Crawford, Laura Castro de Santiago will be in charge of obtaining written consent.
2. The consent process will occur via phone or in a private room with only approved study personnel present. The electronic consent form is structured in different sections including study purpose, study procedures, risks and benefits, additional information (cost, payment, funding, voluntary reminder), privacy protection, stopping the study, and lastly the consent page with signature fields. Each section is a different page, so the interviewee has to advance to the next section. The interviewer will verbally explain each section to the potential participant. By structuring the form in sections, participants receive small amounts of information at a time making it easier to understand. It also creates a natural pause after each section, at which time the interviewer will give the participant the opportunity to ask any questions. After the consent interview, participants will be given the opportunity to think about the decision and discuss privately with their spouse/partner if applicable. If consent interview occurs in-person, the study team member will step out of the room should they want time to think/discuss privately. If the consent occurs via phone, a second phone call will be scheduled to actually sign the consent if they choose to do so.
3. All subjects will be minors, therefore a legal guardian will give informed consent for all subjects.

D. Alternatives to Participation: As stated in the consent form, a parent can choose not to have their child participate.

E. Costs to Subjects: n/a – all study tests and procedures will be provided by the study. No study-related procedures will be billed to insurance. A subject's health insurance status will not impact their ability to participate.

F. How new information will be conveyed to the study subject and how it will be documented: New information will be conveyed to the study subject in writing, via a letter. A dated copy of the letter will be kept in their research record to document that the information was provided. Reconsent will occur if required by the IRB.

G. Payment, including a prorated plan for payment: Subjects will receive $100 per visit attended. The reimbursement will be requested to the ClinCard after each study visit is completed. If a subject chooses to withdraw, they will only receive compensation for the study visit(s) completed. Reimbursement for travel expenses may be available, but will need

to be pre-approved by the PI.

H. Payment for a research-related injury: Treatment for research-related injury will be provided at usual charge. Claims will be submitted to the subject's health insurance policy, government program, or other third party. The subject will be billed for any costs not covered by the insurance.

IV. Data Collection and Protection

A. Data Management and Security:

1. In addition to the PI, Co-I, and IRB-approved study team members, data monitors and auditors from the IRB and regulatory authorities will have access to study data.
2. To maintain confidentiality, subjects will be given a non-identifiable study ID number. Research records containing identifiable information will be kept in Dr. Hull's locked research laboratory. All study records will be retained for 25 years, per university policy for pediatric studies.
3. Subjects will be identified by their study ID number.
4. The study coordinators will maintain and have access to the information that links the subject's identity to their study ID number.
5. Data will be linked to the subject using their assigned study ID number.
6. Data will be stored on the KUMC RedCap server and university P-drive. Only IRB-approved study team members will have access to the data.
7. iPads may be used for electronic data entry during study visits. All data collected via iPad will be entered into RedCap and stored on the KUMC RedCap server. A study specific iPad will be used to take and temporarily save photos of children during the DXA scan. The iPad is passcode protected and only approved members of the study team have access. After each visit, photos will be saved to the university P-drive and deleted from the iPad. If the parent consents yes to the future use of photos, photos will be kept on the P-drive for possible future use in presentations and publications of the research in accordance with the optional multimedia consent and KUMC multimedia authorization form. If the parent consents no to the future use of photos, all photos of the participant will be deleted after sharing them with the participant as a memento of their participation.
8. n/a – no identifiable data will be sent outside KUMC

B. Sample / Specimen Collection: n/a – no samples will be collected

C. Tissue Banking Considerations: n/a – no samples will be collected, therefore no samples will be available to bank

D. Procedures to protect subject confidentiality: There is no appreciable risk of loss of confidentiality. Subjects will be made aware of persons who may see their protected health information in the informed consent document and may choose not to enroll in the study based on the information provided them in accord with 2003 HIPAA regulations. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

E. Quality Assurance / Monitoring

1. All data entry will verified by a second study team member using the original source document to ensure data are accurate, consistent, complete and reliable.
2. n/a – no plans to have ongoing third-party monitoring

V. Data Analysis and Reporting

A. Statistical and Data Analysis: The data analysis plan for each primary aim is described below. There are no planned interim analyses.

1. **Aim 1 analysis:** Determine the relationship between added sugars (grams and % calories) intake during pregnancy and infancy/toddlerhood (birth to 24 months) on brain structure and function. Brain structure will be quantified as brain volume in Freesurfer. Based on prior studies, we will examine the Pearson correlation between hippocampal brain volume and added sugars intake during pregnancy and infancy/toddlerhood. Exploratory analyses will examine correlations with added sugars intake and brain volume in inhibitory control brain regions (i.e., dorsal anterior cortex) and reward related regions (i.e., ventromedial prefrontal cortex). Brain function will be quantified as resting state functional connectivity within the default mode network which includes prefrontal and parietal regions as well as the attention and salience networks. Pearson correlations will examine the relationship between functional connectivity metrics and added sugars intake. Brain function during the EEG Food Go/NoGo task will be quantified as the amplitude of the P3 and N2 components and correlations will examine the relationship between P3/N2 amplitudes and added sugars. Analyses will be corrected for multiple comparisons using a Bonferroni correction.
2. **Aim 2 analysis:** Determine the relationship between fructose (grams and % calories) consumption during pregnancy and infancy/toddlerhood (birth to 24 months) on brain structure and function. As in Aim 1 analyses, brain structure will be quantified as Freesurfer volumes, brain function will be quantified as resting state connectivity, and brain activation during the EEG Food Go/NoGo Task. Correlations will be used to examine the relationship between brain measures and fructose consumption.

B. Outcome: We hypothesize that added sugars and fructose intake will be negatively related to brain structure (hippocampal volume) and function (resting state functional connectivity and P3 amplitude). The strength of the correlations and level of significance will determine if there is a relationship between variables being tested.

C. Study results to participants: Study participants will be informed of the overall study results via a letter, which will be mailed after the study and analysis is complete. Participants will not be given their individual results from study procedures; however, this information may be made available upon request, with approval by the PI.

D. Publication Plan: The present study is a pilot. This project was submitted as a June 2022 R01 and received positive reviews. It will be resubmitted in November 2022 with the hope of receiving R01 funding to extend the study to the entire ADORE GAINS cohort. We hope by getting more participants to complete the cognitive tests and body composition measurements that we will have a large enough sample to publish results relating to those

measurements. Any publications would further support the R01 application.

VI. Literature Cited

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APPENDIX I: VULNERABLE POPULATIONS

I. Cognitively or decisionally impaired individuals: n/a

II. Children: We are targeting children aged 5-6 who previously participated in the ADORE GAINS study. All recruitment and consenting procedures will be targeted toward the parents, as the child will be too young to assent. No child will attain age 18 years while in the study, so no re-consent will be required.

III. Pregnant women: n/a

IV. Prisoners: n/a

V. Students and/or Employees:

- **A.** n/a
- **B.** n/a
- **C.** n/a