Cover Page for Study Protocol

Official Title: The effects of added sugar intake on brain blood flow and hippocampal function in midlife adults

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HUMAN SUBJECTS PROTOCOL

University of Delaware

Protocol Title: The effects of added sugar intake on brain blood flow and hippocampal function in midlife adults

Principal Investigator

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Investigator Assurance:

By submitting this protocol, I acknowledge that this project will be conducted in strict accordance with the procedures described. I will not make any modifications to this protocol without prior approval by the IRB. Should any unanticipated problems involving risk to subjects occur during this project, including breaches of guaranteed confidentiality or departures from any procedures specified in approved study documents, I will report such events to the Chair, Institutional Review Board immediately.

1. **Is this project externally funded?** **X** YES ☐ NO

   If so, please list the funding source: (Pending) National Institutes of Health (NIH) Grant P20 GM113125

2. Research Site(s)

X University of Delaware

☐ Other (please list external study sites)

3. **Project Staff**
Please list all personnel, including students, who will be working with human subjects on this protocol (insert additional rows as needed):

Table 3.1

NAME	ROLE	HS TRAINING COMPLETE?
Christopher Martens, Ph.D.	PI**	Yes
Shannon Lennon, Ph.D.	Co-I	Yes
Curtis Johnson, Ph.D.	Co-I	Yes
Matthew Cohen, Ph.D.	Co-I	Yes
Melissa Witman, Ph.D.	Co-I	Yes
Kevin Decker, Ph.D.	Co-I, Postdoctoral Fellow**	Yes
Faria Sanjana, M.S.	Graduate Student**	Yes
Nick Rizzi, M.S.	Graduate Student**	Yes
Theodore DeConne, B.S.	Graduate Student**	Yes
Fiona Horvat, B.S.	Graduate Student**	Yes
Wendy Nichols, R.N.	Research Nurse	Yes
Kristina Davis	Nutritionist	Yes
Zoe Rigas	Undergraduate	Yes
Ryan Pohlig	Statistician	Yes
Nazim Karaca	REDCap Data Analyst	Yes
Ibrahim Malik	MRI Operator	Yes
Catherine Awad	Research Coordinator**	Yes
Leif Boddie	Undergraduate	Yes
** Indicates that this person has been trained to administer the informed consent.		

4. **Special Populations**

This research does not involve any special populations.

5. **RESEARCH ABSTRACT** Please provide a brief description in LAY language (understandable to an 8th grade student) of the aims of this project.

Aging is the primary risk factor for Alzheimer's disease (AD) which is the most common form of dementia and among the fastest growing causes of morbidity and mortality in the United States. The risk factors for AD emerge during midlife and are similar to cardiovascular and cerebrovascular diseases. The impact of midlife vascular changes on the brain are worsened by poor lifestyle habits, including eating a diet that contains a lot of added sugars (e.g., ultra-processed foods containing high amounts of fructose). One effect of eating a high sugar diet is an elevation in blood triglycerides (TGs), which impairs blood vessel function by causing inflammation; however, it is not known whether eating a lot of added sugars affects the blood vessels in the brain. The purpose of this project is to determine if there is a link between added sugar intake and brain health in midlife adults. Our hypothesis is that eating excess added sugar impairs the structure and function of an area of the brain called the hippocampus by increasing plasma TGs and systemic inflammation. To test this, we will have people eat a high and low sugar diet for 10 days each (in a random order) and test how each diet affects their blood vessel function, the structure of their hippocampus, and their memory performance. We expect to show that eating a diet that contains a lot of added sugars worsens brain health compared to a diet that contains few added sugars. The data generated from this project will help us better understand risk factors for dementia and will be used to support a future grant proposal to the National Institutes of Health aimed at lowering added sugar intake in mid-life adults and individuals with mild cognitive impairment.

6. PROCEDURES Describe all procedures involving human subjects for this protocol. Include copies of all surveys and research measures.

6.1 Study Design

We will conduct a single-blind, randomized-crossover, controlled feeding study (*see Protocol Schema on next page)*. We will provide subjects with 10-days each of a research diet containing low sugar (LS; 5% of energy) vs. high sugar (HS; 25% of energy) in a random order, separated by a 2-week washout. Primary and secondary outcomes will be measured at baseline and at the end of each 10-day intervention.

6.2 Schedule of Activities

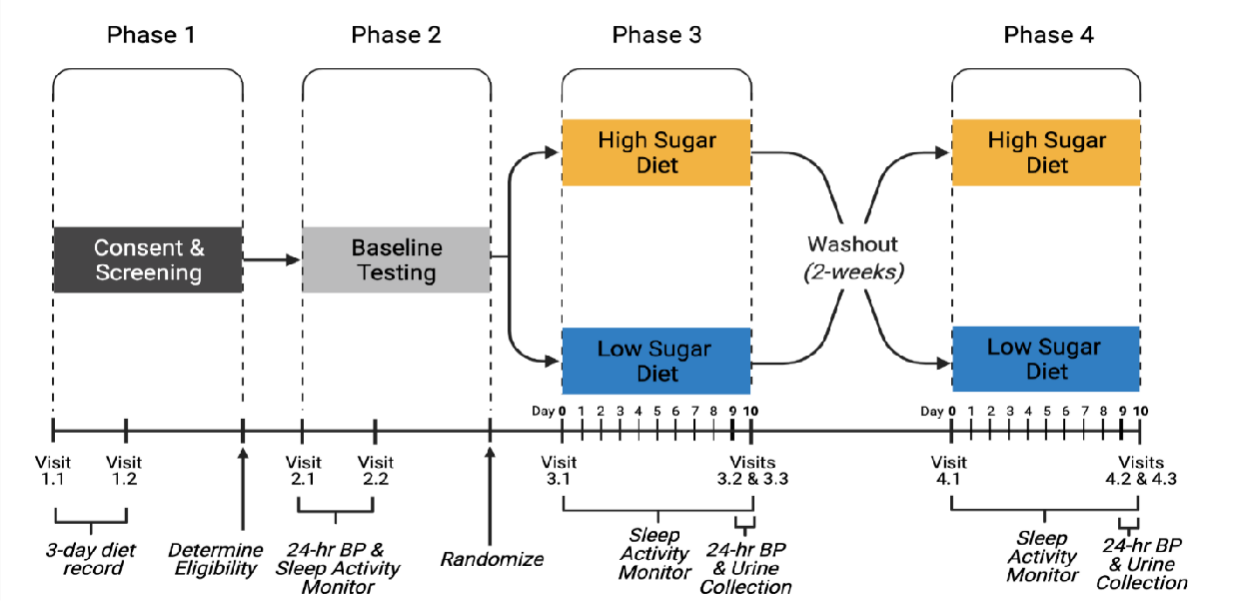
Table 6.1

		Screening		Baseline		Diet #1					Diet #2				
	Day of Diet	NA	NA	NA	NA	0	7	9	10	10	0	7	9	10	10
	Visit Number	1.1	1.2	2.1	2.2	3.1	NA	NA	3.2	3.3	4.1	NA	NA	4.2	4.3
	Duration (hours)	1.5	1.0	1.5	2	0.5	---	---	1.5	2	0.5	---	---	1.5	2
Eligibility Criteria	Informed Consent	•													
	Demographics	•													
	Medical History	•													
	Menstrual History	•													
	PHQ-9 (depression)	•													
	MRI Safety Form	•		•					•					•	
	COVID-19 Form	•	•	•	•	•			•		•			•	
	MAQ	•													
	3-day diet record	®	¬												
	Height		•												
	Body Mass		•			measured at home daily					measured at home daily				
	Body Composition		•							•					•
	Vital Signs		•	•	•					•					•
	Clinical Labs		•							•					•
Control Measures	Family History	•													
	PROMIS Measures	•													
	Sleep Quality	•													
	Food Preferences	•													
	APOE genotyping		•												
	Blood Biomarkers		•						•					•	
	Urine collection		•						•					•	
	IPAQ				•				•					•	
	Sleep Questionnaire				•				•					•	
	Sleep Quality			®	¬	®				¬	®				¬
	Appetite Survey			•		measured at home daily					measured at home daily				
	24-hr urine collection							®	¬				®	¬	
Outcom	24-hr ABPM			®	¬			®	¬				®	¬	
	Cognitive Function				•					•					•
	Blood Vessel Tests				•					•					•
	MRI (CVR, MRE)			•					•					•	

Abbreviations: PHQ-9, Patient Health Questionnaire 9; MRI, magnetic resonance imaging; PROMIS, patient-reported outcome measurement information system; PSQI, Pittsburgh Sleep Quality Index; APOE, apolipoprotein E; ABPM, ambulatory blood pressure monitoring; CVR, cerebrovascular reactivity; MRE, magnetic resonance elastography; MAQ, modifiable activity questionnaire. **Notation:** • indicates measurement will happen on the specified visit; ® ¬ indicates measurement will happen between specified visits.

6.3 Protocol Schema

This schematic depicts the overall study design and outlines the order of key testing visits and outcome measures. Specific details regarding which tests are performed at each visit are described in the **Schedule of Activities** table on the previous page. If a measurement is not performed on a specific visit, due to equipment failure and/or timing issues, the measurement may be repeated on another visit. More specific details regarding each procedure are included below. The purpose of this study is to gain preliminary data for an R01 proposal aimed at improving brain health through dietary modification of added sugars.



6.4. List of Procedures - *The order, frequency and visit(s) at which each measurement will be made is described in the Schedule of Activities table above.*

Eligibility Measures – The following information will be collected to determine eligibility. Some measures may be repeated at other time points as noted in Table 6.1.

Informed Consent - Subjects will meet with the PI or designated member of the study team to review and sign the informed consent form. Individuals trained to administer the informed consent are listed in Table 3.1. The study team member will walk the subject through the consent form and will answer any questions that the subject may have prior to providing their written consent. As documentation of the informed consent, the subject and the investigator performing the consent will sign (or eSign) the informed consent form and a copy of the signed consent form will be given to the subject.

Subject Demographics – A participant intake form will be used to gather basic information including date of birth, sex, race and ethnicity. An equal number of men and women between the age of 50-64 will be enrolled. Race and ethnicity will be gathered for reporting of basic subject characteristics.

Medical History – Subjects will provide information about any chronic medical conditions that they have been diagnosed with, previous hospitalizations and medical procedures, current prescription and over-the-counter medications or supplements, known allergies, health habits (e.g., smoking and alcohol use), and any current medical conditions or symptoms. Subjects diagnosed with a chronic illness or neurological condition will be excluded.

Gynecological History – Because of the unique age-range of this demographic, we recognize that a small proportion of women may still be pre- or peri-menopausal at the time of screening. Because of the small sample size in this initial pilot study and the influence of reproductive hormones on vascular function[1,2], we will only enroll postmenopausal women in this pilot study to reduce potential variability. Female participants will answer questions about their menstrual history to confirm that they are postmenopausal. Menopause status (either surgical or nonsurgical) will be confirmed in all women as a cessation of menses for >1 year; women taking hormone replacement therapy in the preceding year will be excluded from the study[3].

Patient Health Questionnaire 9 (PHQ-9) – A brief, 9-item survey will be used to confirm that subjects do not have major depressive disorder, as this can result in cognitive impairment and impact the primary study results. Subjects must score < 10 on the PHQ-9 to be eligible for this study.

MRI Safety Form – Subjects will answer questions about any implanted metal or devices (e.g., a pacemaker) as well as feelings of claustrophobia to determine if they are eligible to undergo MRI scanning. Because the primary outcomes of this study will be obtained using MRI, subjects must qualify for the MRI scan to be enrolled in this study. We will repeat this form before each MRI scan.

COVID-19 Questionnaire - We will ask subjects about their history with COVID-19, including if they were ever hospitalized or experienced severe symptoms, and details regarding vaccination. Subjects that previously had a severe case of COVID-19 will be excluded from this study. To avoid any influence of an acute inflammatory response on our primary outcomes, we will delay baseline testing on subjects who are planning to become vaccinated until 1 week after their final vaccination dose. We will repeat this form on each day that subjects visit the lab to confirm that there are no changes.

Modifiable Activity Questionnaire (MAQ) - Because high levels of physical activity affect vascular function and brain health, we will administer the Modifiable Activity Questionnaire (MAQ) at baseline and exclude subjects who habitually perform an excessive amount of physical activity.

3-Day Diet Record - Participants will be asked to record everything they eat, including portion sizes, over a 3-day period, including two weekdays and 1 weekend day. This log will be returned during their next screening visit. The study nutritionist will analyze the results prior to enrollment. We will only enroll individuals with a habitual added sugar intake between 10-15% of total energy intake to ensure that our study group is reflective of the average American intake based on the National Health and Nutrition Examination Survey (NHANES)[4].

Height and Body Mass – We will measure body height and mass for reporting of basic subject characteristics and for calculating body mass index (BMI; kg/m^2). Subjects with a BMI < 30 kg/m^2 will be excluded unless their percent body fat is within normal range (see below).

Body Composition – Body composition (% body fat) will be determined using bioelectrical impedance which uses low-level electrical currents through the body to calculate impedance, which is proportional to the amount of body water in adipose tissue. This test will be used primarily as a control variable; however, it may also be used to confirm eligibility based on BMI (as indicated by a % body fat > 25% for men and >33% for women). A tape measure will be used to capture waist circumference.

Vital Signs - Blood pressure and resting heart rate will be measured from the non-dominant arm in the seated and/or supine position after at least 10 minutes of quiet rest using a semi-automated blood pressure device. Repeat measurements will be made (with 2 minutes of quiet rest between recordings) until 3 blood pressure values obtained are within 5 mmHg of one another. These values will be averaged to determine resting systolic and diastolic blood pressure and pulse pressure. Because we are interested in determining the effect of the diets on blood pressure, we will exclude subjects with stage 1 or stage 2 hypertension as defined by a systolic BP ≥ 130 mmHg or a diastolic BP ≥ 90 mmHg.

Screening Blood - Blood will be drawn by the research nurse or trained member of the research team. Screening blood will be sent to LabCorp for analysis of standard clinical blood chemistry including: a comprehensive metabolic panel, hemoglobin A1C, complete blood count with differential, and a lipid panel which will be used for determination of eligibility and for reporting baseline subject characteristics. With the exception of hemoglobin A1C which is not expected to change over the short duration of this trial, these clinical labs will be repeated at the end of each diet to determine if there are any changes in metabolism (see Table 6.2 for complete description of blood markers measured at each visit).

Control Measures – The following information will not be used to determine eligibility but will serve as important control information.

Family History - A family history questionnaire will be used to obtain information regarding family history of cardiovascular diseases, cancer, diabetes and Alzheimer's disease in order to determine if family history may account for any differences between diets.

Patient Reported Outcomes - Subjects will complete the Patient Reported Outcomes Measurement Information System (PROMIS) 57-item questionnaire to assess self-reported feelings about physical function, anxiety, depression, fatigue, sleep disturbance, social engagement, and pain intensity.

Sleep Questionnaires - Self-reported sleep quality will be assessed using the Pittsburgh Sleep Quality Index (PSQI) and the 17-item Munich Chronotype Questionnaire (MCQ). The PSQI is a 19-item self-report tool that assesses healthy sleep metrics over a 1-month time interval. The 17-tiem MCQ determines sleep midpoint for work and free days in the last month.

Sleep Quality - Participants will be provided with an Actiwatch accelerometer watch (Phillips) for an objective assessment of sleep quality. Subjects will be asked to wear the device on their non-dominant wrist for ten days and return it at their next visit.

Physical Activity Questionnaire – Subjects will complete a questionnaire using the International Physical Activity Questionnaire (IPAQ) to determine physical activity measures. The 27 questions of the IPAQ inquiries about physical activity in the last 7 days across 5 domains that relate to occupation, transportation, housework, recreational activity, and time spent sitting,

Food Preference Questionnaire – Subjects will complete a brief questionnaire to determine their food preferences. This information will be used by the nutritionist to design specific diets that are catered to each subject's food preferences to improve compliance and to determine their energy needs.

APOE Genotype – Blood will be sent to LabCorp for determination APOE genotype using a standard clinical PCR test (LabCorp; CPT: 814401).

Blood Biomarkers - Additional blood will be retained by the Neurovascular Aging Laboratory for isolation of serum, plasma and peripheral blood mononuclear cells (PBMCs) which will be used for assessing markers of oxidative stress, inflammation, mitochondrial function, and additional blood lipid factors.

Urine Collection – Urine will be collected for determination of uric acid, which will be used as a marker of adherence to each diet. Urine uric acid is expected to increase during the high sugar diet but not during the low sugar diet.

Home Body Weight and Appetite – Subjects will be provided with an electronic scale and will be asked to record their body mass daily, using an electronic (REDCap) survey that will be emailed to them at the same time each day to confirm that they are consuming the isocaloric diet and not gaining or losing body mass. They will also be asked to rate their perceptions of hunger and satiety using a visual analog scale (0-100 mm).

24-hour Urine Collection – Subjects will collect all of their urine for 24 hours to measure urine sodium concentration as a measure of compliance to each diet.

Outcome Measures – The following measures will be made for testing primary and secondary hypotheses. All measures will be made on the same day (e.g., baseline or day 10 of each diet) with a short break in between arterial stiffness testing to drive to the MRI center.

Visits 2.1, 3.2 and 4.2 (Location: Center for Biomedical and Brain Imaging)

MRI Scan – The MRI scan will include the following tests:

- *Cerebrovascular Reactivity (CVR)* will be measured as the maximal change (from rest) in total cerebral perfusion during 3-minutes of hypercapnia (+9 mmHg increase in $P_{ET}CO_2$) using pseudo-continuous arterial spin labeling (PC-ASL)[6], which uses radio-frequency pulses to magnetically label the water in the blood as an endogenous tracer [6] and thus can be performed without the need for a contrast agent. Hypercapnia will be induced using an MR-compatible prospective end-tidal targeting system which allows for precise and repeatable manipulation of $P_{ET}CO_2$ on a breath-by-breath basis through a face mask using a computerized gas blender (RespirAct™)[7-9]. Beat-to-beat BP will be assessed using an MRI-compatible non-invasive BP (NIBP) monitor (Biopac, Goleta, CA) to normalize blood flow to mean arterial blood pressure.

- *Brain Microstructural Integrity* will be assessed by Magnetic Resonance Elastography (MRE) of the brain. During the acquisition of MRE images, the subject's head will be vibrated using the Resoundant Acoustic Driver System. The Resoundant is an FDA-approved device for inducing small vibrations in a scan subject. The device consists of a small, pillow-like pad that is attached in the MRI head coil and that the subject's head rests on. This pad receives vibrations from tubing that attaches to an active driver placed in the control room. The vibrations received result in a small up-down vibration of the subject's head. The active driver is activated in concert with the MR scanner and is controlled by the investigator performing the scan.

Structural T_1- and T_2-weighted and BOLD MRI images will be acquired following MRE and ASL acquisitions, which are necessary for comparing MRE and ASL findings with brain structure and activity. There will be no vibration during these additional scans. During all scans the subjects will wear disposable earplugs and/or headphones to reduce scanner noise. Participants may also be asked to wear a pulse oximeter on their finger to measure heart rate or a respiratory belt around their chest to measure breathing. This is to observe any potential MRE signal changes from movement associated with breathing or heart rate. Both devices are provided by Siemens with the MRI scanner, are MR-safe and MR-compatible, and interface with the MRI scanner software.

Visits 2.2, 3.3 and 4.3 (Location: Neurovascular Aging Laboratory)

24-Hour Ambulatory Blood Pressure (ABPM) – Subjects will be asked to wear a blood pressure monitor on their upper arm for 24 hours at baseline and during the final day of each diet. The blood pressure cuff will be set to automatically take their blood pressure every 20 minutes during the day and every 30 minutes at night. If possible, subjects will be instructed to sit down and relax when they feel the upper arm blood pressure start to inflate. They will also be asked to refrain from caffeine, alcohol, and exercise on this day.

Arterial Stiffness – The following measures will be made to characterize arterial stiffness.

- *Pulse Wave Velocity (PWV)* – We will use transcutaneous tonometry to measure the pulse in the carotid artery while placing a standard blood pressure cuff around the thigh to measure the pulse in the femoral artery. The pressure wave at each recording site will be aligned to the cardiac cycle

using a 3-lead ECG and PWV will be calculated as the distance between measurement sites divided by the transit time of the arterial pulse wave.

- *Pulse Wave Analysis* – We will derive central (aortic) pulse pressure (PP), augmentation pressure (AP) and augmentation index (AI) from the radial artery pulse wave using applanation tonometry.

- *Carotid artery compliance* – We will record a high-resolution ultrasound video of the carotid artery and use applanation tonometry to determine carotid artery pulse pressure. Compliance will be calculated as the change in carotid diameter for a given change in pressure.

Brain Blood Vessel Test - A small ultrasound probe will be placed on the top of the skin above the temple, and we will record how fast the blood is moving through a major artery in the brain. At the same time, we will measure the amount of carbon dioxide (CO2) in the air that is exhaled by having a soft flexible tube in the front of the nose with ~1 cm extensions that are inserted into the tips of the nostrils and respiration will be measured using a flexible respiration strap. This brain blood vessel test will be measured in response to an acute (~30 second) breath hold, as previously described[5]. The participant will be monitored for signs of distress and the test will be stopped immediately if they express discomfort. The breath-hold test is self-guided and the participant is free to breathe at any time.

Cognitive Function – Memory function will be assessed by performance on the Hopkins Verbal Learning Test (HVLT-R), The Brief Visuospatial Memory Test (BVMT-R), and the NIH Toolbox Pattern Comparison and Flanker tests. The HVLT-T and BVMT-R have multiple versions to prevent learning tests. We will choose three versions of this test at the beginning of the trial and will administer a different version at each visit. The order will be counterbalanced to avoid any effect of test version on cognitive outcomes. The selected NIH Toolbox texts are less prone to learning effects.

Study Restrictions

Subjects must avoid over-the-counter medications for 48 hours, vigorous aerobic exercise or alcohol for 24 hours, and all food, supplements and caffeine for ≥ 12 hours before testing visit but will be allowed to consume water. There are no restrictions for Visit 1.1, unless this visit is combined with Visit 1.2, in which case the above restrictions apply.

Table 6.2. Table of Blood Tests

	Test #	Type	Volume	Visit 1.2	Visit 3.3	Visit 4.3
Screening Labs						
Comprehensive Metabolic Panel	322000	SST Serum	4 mL	X	X	X
Complete Blood Count	005009	EDTA Plasma	4 mL	X	X	X
Lipid Panel	303756	SST Serum	4 mL	X	X	X
Control Measures						
Hemoglobin A1C	001453	EDTA Plasma	4 mL	X	---	---
APOE genotype	504040	EDTA Plasma	4 mL	X	---	---
Blood Biomarkers						
Oxidative Stress/Inflammation	---	EDTA Plasma	4 mL	X	X	X

	---	SST Serum	4 mL	X	X	X
PBMC's	---	EDTA Plasma	32 mL	X	X	X
			Total Per Visit	60 mL (4TBL)	52 mL (3.5 TBL)	52 mL (3.5 TBL)
					Total Volume for Study (mL)	**164 mL (11 TBL)**
					Total Volume for Study (pints)	**1/3 pint**

6.5. Description of Research Diets

The study nutritionist will prepare two 10-day isocaloric research diets for each subject. The diets will differ in the amount of energy from added sugar sources. Added sugars include food products containing syrups and other caloric sweeteners that are added during processing or preparation of food. The majority of added sugars in the United States come from sucrose (table sugar) and products containing high-fructose corn syrup; however other sugars are also used during production or preparation of foods which are considered added sugars. Sugars found naturally in fruits, vegetables and dairy products that have not been added during processing or preparation are not considered added sugars. An example menu of each diet and the corresponding nutrient breakdown is presented in Table 6.3. The specific amount of added sugar in each research diet was selected to maximize the ability to detect changes in vascular function and brain health while also delivering a diet that is representative of current American diets. Data from the National Health and Nutrition Examination Survey (NHANES) suggest that only half of all Americans meet the current the guidelines for added sugar intake[4], which have been set by regulatory bodies including the American Heart Association (AHA), US Department of Agriculture (USDA) and the World Health Organization (WHO) to consume less than 10% of all calories from added sugars[10,11]. Although the average intake of added sugars (as a percentage of total calorie intake) in the United States is ~13%, those who meet current guidelines consume an average of ~5% of total energy from added sugar, whereas the average intake for those not meeting the guidelines is >20%[4]. Accordingly, the chosen amount of added sugar in the low (5% of energy) and high (25% of energy) added sugar diets are within the range of what many Americans are eating and have a high likelihood of modulating blood lipid profiles, thus enabling us to examine the effect of dietary added sugars on important physiological functions indicative of brain health.

Table 6.3. Example Menus

5% Added Sugar	25% Added Sugar
<u>Breakfast</u> Instant oatmeal with 2% milk and raisins Coffee with sugar and cream <u>Snack</u> Popcorn and peanut butter crackers <u>Lunch</u> Turkey sandwich with wheat bread, American cheese and mayonnaise Mini pretzels and apple <u>Snack</u> Tortilla chips, Baby carrots and Hummus	<u>Breakfast</u> Pancakes with syrup Cranberry juice cocktail <u>Snack</u> Coffee Cake <u>Lunch</u> Chicken noodle soup, saltine crackers String cheese <u>Snack</u> Coke-a-cola and Dry roasted almonds,

Dinner Boneless skinless chicken breast, baked potato and green beans		Oreo cookies Dinner Spaghetti and tomato sauce with ground turkey and parmesan cheese	
Nutrient Breakdown			
Carbohydrates (%)	55	Carbohydrates (%)	55
Fat (%)	30	Fat (%)	30
Protein (%)	15	Protein (%)	15
Total Calories	1,944	Total Calories	1,991
Total Carbohydrates (g/day)	271	Total Carbohydrates (g/day)	285
Total Sugar (g/day)	77	Total Sugar (g/day)	157
Added Sugar (g/day)	**25**	**Added Sugar (g/day)**	**131**
Added Sugar (% Kcal)	5	Added Sugar (% Kcal)	25
Fiber (g/day)	26	Fiber (g/day)	12

Diet Adherence

During the strictly controlled diet periods, all meals and snacks will be provided to the subjects. Food for the 10-days will be packed in a cooler and picked up by the subjects at Visits 3.1 and 4.1. All food preparation will be overseen by our registered dietitian and prepared in the metabolic kitchen in the Tower at STAR. Energy needs will be calculated at baseline using the Mifflin-St. Jeor equation[11] and will be corrected for habitual physical activity in order to ensure weight stability throughout the study. Subjects will be asked to consume only the provided food. No other food should be eaten during either of the 10-day diet periods. Subjects will be asked to report extra food items using the "Extra Food Log" form as well as bring back any uneaten food to be weighed. If it is determined that a subject is eating "outside foods" (>50 kcal on 2 or more days), they will be terminated from the study. The total calories provided are designed to keep the subjects weight stable during this period (this is referred to as "isocaloric"). We will also provide them with water (bottled) to drink during each diet. Subjects will report to the lab on the 10th day of each diet to undergo all post-intervention testing (Visits 3.2, 3.3, 4.2, & 4.3).

Washout Period

Subjects will complete a 2-week washout period in between each diet to ensure enough time for lipid markers and physiological functions to return to baseline values between diets. Previous dietary intervention crossover studies over a 3-6 week period *without* a washout period have **not** reported carryover effects on measures of large elastic artery stiffness, vascular function or cognitive performance[13,14]. Accordingly, we expect the proposed measures of physiological function to return to baseline quickly and the proposed 2-week washout period represents a conservative approach.

7. **STUDY POPULATION AND RECRUITMENT**

Study Population – The target enrollment for this study is 200 healthy adult men and women between 50-64 years of age, with a target completion of 40 participants. This age group has been defined by NIH as being at high risk of accumulating cardiometabolic risk factors that preclude chronic age-related diseases[15-17]. To avoid any influence of baseline diet (e.g., chronic consumption of a high-sugar diet prior to enrollment), will only enroll those with a habitual intake of added sugar that is reflective of the average American intake (~13% of total calories) based on the National Health and Nutrition Examination Survey (NHANES)[18]. This will increase the likelihood of observing changes in our primary outcome variables in response to the proposed research diets which are designed to deviate from baseline proportion of added sugars by about 10% of calories in each direction. Importantly, even if

subjects are close to the upper or lower cutoff, we are still likely to observe changes in primary outcomes when placed on the opposite arm of the crossover. Because this is a pilot study, we will analyze the data after the first 5-10 participants have completed the trial and ensure that we are seeing an effect of the diets on the primary outcome variables. If we do not see an effect, we will amend the protocol to increase the duration and/or modify the composition of the diets.

To be eligible to participate, volunteers must meet the following specific enrollment criteria:

Inclusion Criteria:

- ability to provide informed consent;
- men and postmenopausal women aged 50-64 years;
- habitual intake of added sugars ≤15% of total calories[18];
- systolic BP < 130 mmHg; diastolic BP < 90 mmHg[19];
- body mass index (BMI) <30 kg/m^2 and % body fat < 25% for men and < 33% for women
- fasting triglycerides < 200 mg/dl (< 2.3 mmol/L)[20];
- LDL cholesterol <160 mg/dl (4.14 mmol/L) [21]
- fasting plasma glucose <126 mg/dl (<7.0 mmol/L) and hemoglobin A1C < 6.5% at screening[22];
- weight stable in the prior 6 months (≤ 2 kg weight change);
- blood chemistries indicative of normal liver enzymes and renal function (estimated glomerular filtration rate using the MDRD prediction equation must be >60 ml/min/1.73 m^2)

Exclusion Criteria:

- current use of medications or supplements known to lower blood triglycerides or cholesterol (e.g., fibrates, statins, high dose niacin, high dose omega-3 supplement);
- chronic clinical diseases (e.g., coronary artery/peripheral artery/cerebrovascular diseases, heart failure, diabetes, chronic kidney disease requiring dialysis, neurological or autoimmune conditions affecting cognition (e.g. Alzheimer's disease or other form of dementia, Parkinson's disease, epilepsy, multiple sclerosis, large vessel infarct);
- major psychiatric disorder (e.g. schizophrenia, bipolar disorder);
- major depressive disorder (PHQ-9 ≥ 10);
- current or past (i.e., last 3 months) use of anti-hypertensive or other cardiovascular-acting medications known to influence vascular function and/or arterial stiffness[23.24];
- current medication use likely to affect CNS functions (e.g. long active benzodiazepines);
- concussion within last 2 years and ≥ 3 lifetime concussions[25];
- heavy alcohol consumption (defined by the CDC and USDA as ≥8 drinks/week for women and ≥15 drinks/week for men)[26].
- claustrophobia, metal implants, pacemaker or other factors affecting feasibility and/or safety of MRI scanning;
- recent major change in health status within previous 6 months (i.e., surgery, significant infection or illness);
- current smoking within the past 3 months.
- High degree of physical activity as defined by ≥ 25 leisure MET-hours/week, within the past 3 months.

Recruitment

Subjects will be recruited from Newark, DE and the surrounding area. Recruitment methods may include posted flyers, digital advertisements (e.g., Facebook), direct mailings to age-specific regional mailing lists, UD alumni and employee mailing lists, UD press releases, information sharing with a

variety of minority organizations associated with UD and the Newark community, participation in health fairs, and emails sent to past participants interested in being recontacted about new research studies.

Primary Exit Criteria

- Completion of the study;
- Failure to eat the diet during the dietary trial;
- Eating foods other than those prepared for the subject during the dietary intervention (>50 kcal/day on 2 or more days);
- Not showing up to the agreed upon study visit two times or chronically canceling within 2 hours of the agreed upon visit (two times);
- Failure to complete MRI scanning (e.g., due to claustrophobia);
- Donating blood or a large volume of blood drawn within 6 weeks of study participation or during the study (subjects should not donate blood in 6 weeks following study participation).

Subjects will also have the option of withdrawing from the study at any time for any reason. The number of subjects exiting the study and the reasons for exit will be carefully documented. Subjects who withdraw from the study will be replaced in order to achieve the sample size required for appropriate statistical power.

8. **RISKS AND BENEFITS**

List all potential physical, psychological, social, financial or legal risks to subjects (risks listed here should be included on the consent form).

High Sugar Diet – There is minimal risk associated with consuming a high sugar diet for this duration. Each diet will be specifically catered to each subject, taking into account food preferences and any known food allergies. The diets are designed to be isocaloric and are not expected to change body weight. In the event of reported weight loss or reports of hunger, diets will be adjusted to increase energy intake. Unlike high amounts of glucose, 90% of dietary fructose intake is metabolized by the liver and does not substantially increase blood glucose levels[27]; however, we do expect the high-sugar diet to raise plasma TGs and there is a possibility that it will also raise other cardiometabolic risk factors (e.g., LDL-cholesterol)[28-29]. We expect these parameters to return to baseline after participants return to their habitual diet. Moreover, there is no evidence that the short-term dietary changes proposed in this study influence long-term risk of cardiometabolic disease or cognitive impairment. Importantly, our high-sugar diet is representative of what a large number of adults in the United States are already consuming on a daily basis[4,18].

Venous Catheter/blood draw – Discomfort associated with insertion of the needle; local bleeding and a small hematoma (~10% of cases); risk of blood clot (~1% of cases); risk of infection of a hematoma or significant external blood loss (<1 in 1000); risk of fainting.

Genetic Risk for AD – One of the blood tests will determine whether or not the subject carries a gene that has been associated with risk for Alzheimer's disease (AD). People with this gene have a higher chance of developing AD compared with people who do not carry the gene. Some people who carry the gene may never develop AD. Others may develop AD even though they do not carry the gene. Subjects will be provided with the results of this test upon request only.

24-hour ABPM – There are no known risks associated with wearing the 24-hour blood pressure monitor. Subjects may find it irritating as it inflates regularly.

Acute Hypercapnia (CO_2 inhalation) – There are no risks associated with inhalation of small amounts of carbon dioxide. The hypercapnia protocol has been used in human studies clinically and in the research setting for decades without any adverse events. Study subjects may experience an increase

in heart rate, flushed skin, minor disorientation, or develop a mild headache during hypercapnia. Some subjects may notice a slight metallic taste when breathing the CO_2 gas mixture.

MRI Scan – MRI is an imaging technique that uses radio waves and magnetic fields to produce images of internal structures in the body. Unlike X-rays, the MRI does not use any ionizing radiation, and it does not use radioactivity, so there is no radiation related risks from having an MRI scan done. Below there is a description of MRI related risks and what is being done to reduce any possible risks associated with them:

- Metal – The MRI scanner produces a constant strong magnetic field, which may cause any metal implants, clips, or implanted medical devices within the body to shift position or malfunction. Subjects will be screened prior to entering the MRI scanner and those with implanted metal, clips or devices that are not MR-compatible will be excluded from this study. Metallic objects brought into the MRI environment can become hazardous projectiles and can also interfere with the data quality. To minimize this risk, subjects will be asked to remove all metal jewelry, belts with metal buckles, and all items from the pockets, including coins, electronics (including cell phones and hearing aids) and wallets. Some subjects may be asked to change into a gown if their clothing contains significant metal, including metal underwire bras.

- Inner ear damage – MRI scanning produces loud noises that can cause damage to the inner ear if appropriate hearing protection is not used. Subjects will wear earplugs and headphones to protect their ears.

- Claustrophobia – The subject's head will be centered inside a close-fitting scanning coil positioned in the bore of the scanner. If the subject feels anxious in confined, spaces they may not want to participate. If they are unsure, they can try the "mock" scanner at the MRI facility to evaluate their comfort level with the enclosed space of the magnet bore. If they decide to participate and begin to feel claustrophobic, they will be able to communicate with the research team via the intercom or the squeeze ball and the test will be discontinued.

- Burns – In rare cases, contact with the MRI transmitting and receiving coil, conductive materials such as wires or other metallic objects, or skin-to-skin contact that forms conductive loops may result in excessive heating and burns during the experiment. The operators of the MRI scanner will take steps, such as using foam pads when necessary, to minimize this risk. Tattoos with metallic inks can also potentially cause burns. Subjects will be instructed to let the MRI operator know immediately if they experience any heating or burning sensations during a scan. The scanning session will be stopped as soon as they tell the operator.

- Nerve or muscle stimulation – While the scanner is operating, there is a small chance that the rapidly changing magnetic fields could cause a slight tingling sensation or a muscle twitch, usually felt in the upper arms or torso. While these sensations may be startling, they are not dangerous or a health risk, and they have no lasting consequences. The sensations should stop when the scan ends. Because these sensations may nevertheless be distracting or even possibly uncomfortable, subjects will be instructed to squeeze the signal bulb to alert the scanner operator if they feel uncomfortable tingling or muscle twitching and we will immediately stop the scan. They will then have the opportunity to withdraw from the study or to continue.

- Magnetic Resonance Elastography*:* This study uses elastography to study the mechanical properties of the brain. Elastography uses low-amplitude (tens of microns) vibration at 50 Hz applied to the head to generate shear waves in the brain. Vibration is applied for less than 10 minutes of total duration. There is no known risk to vibration exposure at this amplitude, frequency, and time. However, it is possible the vibration may cause the subject to feel slight discomfort, and they will be instructed to alert the technologist via intercom or by squeezing the emergency ball if this occurs. The scan and vibration will be stopped immediately.

In your opinion, are risks listed above minimal* or more than minimal? If more than minimal, please justify why risks are reasonable in relation to anticipated direct or future benefits.
*(*Minimal risk means the probability and magnitude of harm or discomfort anticipated in the research are not greater than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests)*

The risks associated with all procedures in this protocol are minimal and the magnitude of harm or discomfort are not greater than those encountered during similar procedures completed as part of routine clinical care (e.g., phlebotomy, MRI scanning). Although patients receiving a clinical MRI scan do not typically undergo the MRE and hypercapnia protocols, the overall risks and discomforts associated with these procedures are extremely low.

What steps will be taken to minimize risks?

The potential risks of the proposed study will be minimized by:

- using only safe, well-established procedures with only qualified and experienced personnel performing the procedures;
- having a registered dietician prepare and package all food in a dedicated research kitchen;
- monitoring any gastrointestinal discomfort associated with consuming the diet;
- ensuring constant personal monitoring of each experimental session by the investigators;
- screening all participants for ability to undergo an MRI;

Describe any potential direct benefits to participants.

Because the risks of participating in this study are relatively small, the risk-to-benefit ratio also is relatively small. Subjects will receive benefits associated with overall knowledge of their health from the extensive testing performed for screening purposes and the established subject characteristics (i.e., blood glucose status, plasma lipids and lipoprotein profile, blood pressure, blood chemistries).

Describe any potential future benefits to this class of participants, others, or society.

The findings from the proposed research will provide important new information regarding the influence of dietary intake of added sugar on cardiovascular and brain health in midlife adults. In addition, the proposed research will also provide important insight into the potential mechanisms (e.g., TGs, inflammation) by which added sugar may negatively impact brain health. This information will contribute to the knowledge of how to prevent and treat cerebrovascular and cognitive impairment in midlife adults and may lead to future insight into preventing Alzheimer's disease risk.

If there is a Data Monitoring Committee (DMC) in place for this project, please describe when and how often it meets.

There is no DMC for this study.

9. **COMPENSATION**

Will participants be compensated for participation? Yes

If so, please include details.

Subjects will receive $200 in total compensation for completing this study. They will receive $100 for completing the first diet and $100 for completing the second diet (including the laboratory visits). If they drop out after the screening visit (Visit 1.2), they will not be compensated because subjects will be receiving medical information from all of the screening tests free of charge. If they consume the prepared food and complete the 24-hour blood pressure monitoring but do not complete the laboratory visit, they will receive half the amount for that diet trial ($50); however, if this occurs during the first diet, the investigators reserve the right to terminate the subject depending on the reason for not completing the laboratory visit. Compensation does not include travel time, but upon request will compensate subjects for their travel mileage, at the current IRS approved mileage rate. Compensation is based on completion of study visits, not individual procedures. Subject can be compensated an additional $20 for each subject referral that is consented into this study. Table 10.1 outlines the total compensation for each visit is presented below.

Table 10.1 Compensation Schedule

	Visit #	**Duration**	**Purpose**	**Total**
Phase 1	Visit 1.1 & 1.2	2.5 hr	Informed Consent & Screening Measures	No compensation Subjects receive free health information
Phase 2	Visit 2.1	2 hr	Vascular and Cognitive Function	$50 (only if visit 3.2 & 3.3 is not completed)
	Visit 2.2	1.5 hr	MRI	
Phase 3	Visit 3.1	30 min	Diet Pick-Up	$100
	Visit 3.2	2 hr	Vascular and Cognitive Function	
	Visit 3.3	1.5 hr	MRI	
Phase 4	Visit 4.1	30 min	Diet Pick-Up & Blood Draw	$100
	Visit 4.2	2 hr	Vascular and Cognitive Function	
	Visit 4.3	1.5 hr	MRI	
Total Duration		14 hr	**Total Compensation**	**$200**

10. **DATA**

Will subjects be anonymous to the researcher? No.

If subjects are identifiable, will their identities be kept confidential? (If yes, please specify how)

Subjects will not be individually identified, except by a subject ID number known only to the investigators.

How will data be stored and kept secure (specify data storage plans for both paper and electronic files. For guidance see http://www.udel.edu/research/preparing/datastorage.html)

Physical data will be stored in a locked file cabinet in the PI's lab space and will only be accessible by

members of the research team. Physical data that are transcribed into an electronic database, and electronically captured data will be kept on a password protected laboratory server that is maintained by the University of Delaware. Electronic data will be periodically backed-up onto an external hard drive that will be kept in a locked office space. All of the ultrasound images and files generated by the SphygmoCor and RespirAct devices will be coded with an identification number and will not contain the name of the subject. The names of subjects will not be identified in any publication arising from these studies. Only the PI and research staff will have access to data from this study.

How long will data be stored? All data will be stored in a locked cabinet or a password protected computer indefinitely.

Will data be destroyed? ☐ YES **X** NO (if yes, please specify how the data will be destroyed)

Will the data be shared with anyone outside of the research team? **X** YES ☐ NO (if yes, please list the person(s), organization(s) and/or institution(s) and specify plans for secure data transfer)

The data may be shared with the National Institutes of Health. The trial will be registered with clinicaltrials.gov once the funding is finalized; however, only de-identified data will be reported.

How will data be analyzed and reported?

Data will be aggregated and reported as group means or as de-identified, individual data points. Because this study is intended to support a larger R01 proposal, we will analyze the data after the first 5-10 subjects have completed all testing. If we do not observe any effect of the diet on primary outcomes, we will consider amending the protocol to increase the duration of the controlled feeding window and/or modify the composition of the diets. Any subjects tested prior to a major amendment of the protocol will be replaced to achieve the target sample size.

11. **CONFIDENTIALITY**

Will participants be audiotaped, photographed or videotaped during this study?

Subjects will be audiotaped for the purpose of analyzing their memory tests after each visit. These recordings will be deleted immediately after the test has been scored.

How will subject identity be protected?

Information obtained from this study will be kept strictly confidential. Subjects will not be individually identified, except by subject number, known only to the investigators. All data stored as paper files or digitally will be kept indefinitely. The paper files are stored in a locked cabinet. While the results of the research may be published, subjects' names and identities will not be revealed.

Is there a Certificate of Confidentiality in place for this project? (If so, please provide a copy).

This study is (pending) funding from NIH which automatically issues a CoC per the notice of award.

12. **CONFLICT OF INTEREST**

(For information on disclosure reporting see: http://www.udel.edu/research/preparing/conflict.html)

Do you have a current conflict of interest disclosure form on file through UD Web forms?

Yes.

Does this project involve a potential conflict of interest*?

* As defined in the University of Delaware's Policies and Procedures ,a potential conflict of interest (COI) occurs when there is a divergence between an individual's private interests and his or her professional obligations, such that an independent observer might reasonably question whether the individual's professional judgment, commitment, actions, or decisions could be influenced by considerations of personal gain, financial or otherwise.

If yes, please describe the nature of the interest:

13. **CONSENT and ASSENT**

__X__ Consent forms will be used and are attached for review (see Consent Template under Forms and Templates in IRBNet)

____ Additionally, child assent forms will be used and are attached.

____ Waiver of Documentation of Consent (attach a consent script/information sheet with the signature block removed).

____ Waiver of Consent (Justify request for waiver)

14. **Other IRB Approval**
Has this protocol been submitted to any other IRBs?

If so, please list along with protocol title, number, and expiration date.

15. **Supporting Documentation**
Please list all additional documents uploaded to IRBNet in support of this application.

Informed Consent Form

Screening and Control Measures – *the uploaded forms are examples only and the format may be modified for online administration via REDCap.*

- COVID19 Form
- Daily Appetite Survey
- Diet Recall Form
- Family History
- Food Preference Form
- Intake and Demographics Form
- International Physical Activity Questionnaire (IPAQ)
- Medical History Form

- Menstrual History Form
- Modifiable Activity Questionnaire (MAQ)
- MRI Screening Form
- Munich Chronotype Questionnaire
- Pittsburgh Sleep Quality Index (PSQI)
- PHQ-9 Depression Survey
- PROMIS-57 Profile v2.1

Outcome Measures – *the uploaded forms are examples only and the format may be modified for online administration via REDCap or using an iPad (e.g., NIH Toolbox)*

- Brief Visuospatial Memory Test (BVMT-R)
- Hopkins Verbal Learning Test (HVLT-R)
- NIH Toolbox
    - Pattern Comparison Processing Speed Test
    - Flanker Inhibitory Control Test

Advertisements

- novaLab_Sugar Feeding Study_ Email and Mailed Letter
- novaLab_Sugar Feeding Study_Recruitment Flyer
- novaLab_Sugar Feeding Study_Udaily Bulletin & Social Media Ad

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