INSTRUCTIONS:

- *Use this "TEMPLATE PROTOCOL (HRP-503)" to prepare a study protocol outlining your research plan.*
- *Depending on the nature of your study, some major sections might not be applicable to your research. If so, simply mark as "N/A." For example, a simple survey might have many sections with "N/A." For subsections (e.g., 1.x or 8.x) you can mark as "N/A"if you are certain that the subsection is not applicable.*
- *Once the IRB/HRPP approves your submission, your latest approved version of the protocol will be stored in the IRB Protocol Management online system.*
- *If your research plan changes and you need to modify the protocol, please submit an amendment to Protocol Management with the requested modifications. Download your current protocol from Protocol Management and indicate the changes/revisions using the track changes feature in order to make review of the modifications easier to follow. If you are unable to use track changes, please create a new paragraph wherever you need to make a change, and indicate "Amendment: Date" before making a change to any section. Protocol management will store the older versions of your protocol if the IRB or HRPP staff need to compare them during the review.*

PROTOCOL TITLE:

Include the full protocol title.

The impact of physiological response to sugar on brain activity and behavior

PROTOCOL NUMBER: #23-297

Include the number assigned in Protocol Management (verify this has been added before submitting protocol to HRPP).

Click here to provide a response.

PRINCIPAL INVESTIGATOR:

Full Name and Degrees: Alexandra Gold DiFeliceantonio, PhD
Department: Human Nutrition, Foods and Exercise
Telephone Number: 540-526-2285
Email Address: dife@vt.edu

FUNDING:

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Funded already or in the proposal phase?: funded
Is Virginia Tech the primary awardee or the coordinating center of this grant or contract? If not, list the primary institution: VT

VERSION NUMBER/DATE:

Include the version number and date of this protocol. Versions should start at 1.0.

1.0

REVISION HISTORY:

Use this table to keep track of changes. Add more rows as needed.

Revision #	Version Date	Brief Summary of Changes (i.e., the different sections)	Consent Change?
1	4/3/23	1. registered on clinicaltrials.gov 2. revised sections 8.2, 17.1, 17.2, 3. updated consent forms 4. updated Interest in Study Emails (supporting docs) 5. added participant results materials (supporting docs)	yes
2	4/7/23	1. finished sentence in 17.2	no
3	4/10/23	1. updated sections 12.1 and 12.2 in protocol to reflect supporting documents	no
4	12/13/23	1. Updated recruitment information for pilot study to include new methods of recruitment (section 6.0) 2. Provided more general ingredients used in drink stimuli since we don't know which non-nutritive sweeteners will be used yet (that is the purpose of the pilot study), but all ingredients being tested are regularly available in the US food supply and have likely been consumed by nearly every participant. (section 7.1) 3. Removed blood pressure and BIA measurements since we no longer have this equipment available to use (section 8.2) 4. Changed timing of when dietary, physical activity, and health behavior questionnaires occur; also removed information about contacting lab personnel for opting out of further participation, as this this is not needed or necessary when participants complete the questionnaires at a session in which they are	yes

		already interacting with study staff. (Section 8.2) 6. Changed language describing blood collection tubes, as we are experiencing challenges in types/sizes available for procurement at different times (section 8.2) Added perceptual ratings to at-home/online session (section 8.2) 7. Added information about checking in with participant before removing from MRI environment after a squeeze bulb activation (section 8.3) 8. Removed "blood will not be drawn from the same site during any 1 session" as this is not in line with IV catheter placement (section 8.3) 9. Removed information about providing participants with separate, single-use blood collecting devices, as these are performed in our blood collection laboratory by research study staff (section 8.3) 10. Added general "other blood metabolites" to data collected during study (section 8.4) 11. Changed "energy expenditure" to "metabolic" to be more accurate when describing location of data storage (section 9.1) 12. Removed excess and unnecessary information about Ripple storage of personally identifiable information being separate from research data (since we use a completely separate software and process for research data storage) (section 9.4) 13. Changed inclusion/exclusion criteria: BMI up to 27, removed birth control as exclusion, removed contraindications for BIA (section 12.2) 14. Added 1/2 hour to time involvement of consent session in all relevant places (protocol, session reminder emails, consent from) 15. Changed MRI risk to include other ear protection instead of headphones, as the head coil system used for this study is not large enough to fit headphones for most participants; also noise reduction rating of the ear plugs, as we are able to procure different ones at different times based on supply (section 8.2, 8.3, 17.1)	

5	4/1/24	1. Removed personnel: Amber Burns, Ryann Kolb 2. Added personnel: Rhianna Sullivan, Abby Valle, Han Lee, Lara Tablieh 3. Section 8.2: Changed pilot study session length to ~2 hours; changed protocol to allow participants to complete as many pilot study sessions as they would like. 4. Section 11.1: Changed pilot study length to ~2 hours 5. Section 15.4: Changed pilot study length to ~2 hours	Yes

Table of Contents

1.0 Study Summary

Study Title	The impact of physiological response to sugar on brain activity and behavior
Study Design	This is a within-subjects study design. Results will be analyzed using linear regression and linear mixed effects models.
Primary Objective	The primary objective of this study is to study the effects of different types of sugars/carbohydrates commonly and abundantly found in the US food supply on metabolic rate, blood metabolites, and food reward.
Secondary Objective(s)	There are no secondary aims for this study.
Study Population	The general US population
Sample Size	20 for the pilot flavor tasting; 200 for the full study
Research Intervention(s)/ Investigational Agent(s)	Blood measurements, resting and post-consumption energy expenditure, questionnaires and ratings, fMRI
Study Duration for Individual Participants	Screening session: 2 hours In-Person Sessions: 3 sessions of 4 hours each, 12 hours total Online Sessions: 3 sessions of 15 minutes each, 45 minutes total Take-Home Drinks: 4 sessions of 5 minutes, 20 minutes total Post-test session: 3 hours
Acronyms and Definitions	fMRI, functional magnetic resonance imaging; FBRI, Fralin Biomedical Research Institute; CHBR, Center for Health Behaviors Research; SSB, sugar sweetened beverage; HNL, Human Neuroimaging Lab; ELISA, Enzyme linked immunosorbent assay

2.0 Objectives

2.1 Describe the purpose, specific aims, or objectives of this study:

The purpose of this study is to test the influence of different types of carbohydrates on metabolic response and food reward.

2.2 State the hypotheses to be tested:

For aim one, our hypothesis is that sucrose conditions greater appetitive effects than either fructose or glucose, its constituents, alone. For aim 2, our hypothesis is that fructose, incorporated into high fructose corn syrup, enhances the reinforcing properties of glucose, leading to more appetitive responses than sucrose paired with a non-nutritive sweetener, or sucrose alone.

3.0 Background

3.1 Summarize the relevant prior research on this topic and gaps in current knowledge within the field of study:

On any single day, 63-80% of people consume a sugar sweetened beverage (SSB), and in the US, adults consume ~46% of total added sugars in the form of SSBs. Beyond the established links with weight gain and increased adiposity deposition, excessive sugar intake is associated with reduced insulin sensitivity and may increase risk for type 2 diabetes. Fructose has independently been associated with increased intrahepatic lipid content and increased risk of non-alcoholic fatty liver disease. So, reducing SSB consumption could be an effective tool to reduce rates of obesity, type 2 diabetes, an nonalcoholic fatty liver disease. The assumption has been that oral sweet tastes drives SSB consumption; however, if sweetness alone drive consumption, the advent of "diet" drinks, which contain artificial sweeteners, would have driven substantially reduced SSB intake. This is not the case, and the exact reasons are unclear. We propose a potential underexplored mechanisms" engagement of post-oral/postingestive metabolic pathways that support behavior and preference that are not engaged by artificial sweeteners. Recent data indicated that post-ingestive signals of sugar availability may contribute to food reward and choice, and nutrients in the gut are able to rapidly change bran activity and influence food choice and behavior. These gut-brain circuits, however, are not well-understood. Furthermore, most of this work in understanding gut-brain connections has been performed in animal models; translating these findings to humans is necessary.

3.2 Describe any relevant preliminary data:

This is a new project without preliminary data collected.

3.3 Based on the existing literature, provide the scientific or scholarly rationale for and significance of your research and how will it add to existing knowledge:

Prior studies in humans indicate that while energy expenditure response is similar after consumption of equal amounts of fructose, glucose, and sucrose (a dimer of glucose + fructose), carbohydrate oxidation and blood glucose responses differ. Elevated carbohydrate oxidation responses appear to be driven by the presence of fructose, and elevated blood glucose responses appear to be driven by the presence of glucose. Prior work also suggests that post-ingestive signals of glucose availability, measure specifically as blood glucose levels, intestinal glucose transporter activity, and carbohydrate oxidation rate, are all associated with elevated brain response to calorie-predictive flavor cues and reward learning of these flavor cues. However, in animal models, glucose has been shown to repeatedly and reliably condition these calorie-predictive learning responses, but fructose does not. Human work has indicated that oxidation of glucose is critical for these responses. Thus, it is unclear what roles fructose and glucose each play in conditioning reward responses and flavor-calorie learning. We hypothesize that fructose plays a synergistic role in enhancing flavor-calorie learning without itself conditioning the reward response. We will test this hypothesis through this protocol.

4.0 Study Endpoints

*4.1 Describe the primary and secondary **study** endpoints. See links below for discussion of study endpoints and how they may differ from study objectives. These are most common in clinical trials but are sometimes applicable to other types of biomedical research, as well as social, behavioral, or educational research. See link below for a discussion.*

https://docs.google.com/document/d/1Wocz7K7a0hCQJPPO_khh5l1SQQjhGDDGHzcOPRHR5Tw/edit?usp=sharing

Primary study endpoints are indirect calorimetry measurements, blood glucose measurements, beverage condition ratings and preferences (behavioral measures), and brain activation measured by fMRI.

*4.2 Describe any primary or secondary **safety** endpoints. These should be included for all studies that are greater than minimal risk. (Minimal risk: The probability and magnitude of harm or discomfort anticipated in the research that are not greater in and of themselves than those ordinarily*

encountered in daily life or during the performance of routine physical or psychological examinations or tests.):

There are no safety endpoints.

5.0 Study Design and Statistical Analysis Plan

5.1 Describe the basic study design/approach (e.g., qualitative study using five focus groups of first year students to describe assimilation into the university community; randomized controlled trial of a behavioral change intervention to increase dietary intake of whole grains; pre- post-test evaluation of new pedagogical techniques to improve adult literacy):

The pilot study will be a within-subjects design used to compare ratings of beverages with different levels of sweetness provided by different sugars and non-nutritive sweeteners (see 7.0:Investigational Agents: Beverage Solutions, below, for ingredients).

The overall study will be a within-subjects (crossover) design used to compare behavioral (beverage ratings and preferences), metabolic (blood glucose & metabolites, indirect calorimetry measures), and brain activation responses (fMRI) to glucose-, fructose-, and sucrose-containing beverages (Aim 1); and sucrose-, high fructose corn syrup (HFCS)-, and non-nutritive sweetener + sucrose- containing beverages (Aim 2).

5.2 Describe corresponding data analysis plan/approach (e.g., content analysis of focus group transcripts; descriptive analysis followed by linear regression modeling; nonparametric analysis of pre- and post-test measures):

Linear mixed effects models will be used to assess the following outcome variables: change in indirect calorimetry measures, change in blood glucose and insulin, change in beverage liking. Linear regression will be used to relate blood and indirect calorimetry measures to liking and other subjective ratings.

6.0 Setting

6.1 Describe the sites or locations where your research team will conduct the research. Consider each of the items listed below:

- *Identify where your research team will identify and recruit potential subjects.*
- *Identify where the team will perform the research procedures.*

- *Describe the composition and involvement of any community advisory board(s).*
- *For research conducted in other locations, describe:*
  - *Site-specific regulations or customs affecting the research at those locations.*
  - *Local scientific and ethical review structure at those locations. Examples include work in other cultures or ethnic groups (within or outside of the U.S.) and work with churches. The HRPP will provide additional guidance for international research.*

Pilot Study:
A convenience sample of individuals will be recruited through word-of-mouth, posted flyers, social media, and other recruitment avenues. Individuals who are willing and able to come to the Center for Health Behaviors Research (CHBR) at the Fralin Biomedical Research Institute (FBRI) will be invited to participate. All study procedures for the pilot study will be conducted at the CHBR at FBRI.

Full Study:
Recruitment will be done through flyers posted in the local community and social media posts. Both avenues of participant recruitment will include information about the study as well as contact information (i.e. email and phone number) for the study and instructions to access the screening survey. Individuals within our Ripple (HIPAA-compliant, see information below in section 9.4) database who have previously indicated they would like to be considered for future studies will also be contacted.

The study procedures for the in-person visits will all take place at the Fralin Biomedical Research Institute (FBRI) at VTC in Roanoke, VA. The consent session will occur at the Center for Health Behavior Research (CHBR) at the FBRI or in metabolic core space in Riverside 4 at the FBRI at VTC. Indirect calorimetry blood draw sessions will occur in the metabolic chamber at the FBRI at VTC. The MRI scan will take place in the Human Neuroimaging Lab (HNL) at the FBRI.

7.0 Study Intervention(s)/Investigational Agent(s)

7.1 Describe the study interventions (including behavioral interventions) and/or investigational agents (e.g., drugs or devices) to be used in this study. Consider each of the items listed below:

- *Drug/Device Handling: If the research involves drugs or devices, describe your plans to store, handle, and administer the drugs or devices so that they will be used only on subjects, and only by authorized investigators.*

- *Describe whether any of the following will be used: microwaves, X-rays, DEXA scans, general anesthesia, or sedation*
- *If control of the drugs or devices used in this protocol will be accomplished by following an established, approved organizational SOP (e.g., Research Pharmacy SOP for the Control of Investigational Drugs, etc.), please reference the SOP in this section.*

Beverage Solutions:
Participants will consume different beverages and "tasteless" solutions at different times throughout the study. Ingredients used in the solutions include: food-grade table sugar, high fructose corn syrup, dextrose, fructose, -non-nutritive sweeteners that are commonly found in the US food supply, citric acid, potassium chloride (a common salt), and sodium bicarbonate (baking soda). Beverages will also include flavors and food coloring commonly used in commercial food products.

All ingredients are common food additives that nearly every participant would have consumed previously in their normal diet.

Device Handling:
This research involves the use of a metabolic chamber which is located in a restricted space requiring proxy access. The MRI scanner involved in this work is located in a restricted, proxy access behind biometrically secure doors

7.2 List the name of all drugs (including any vitamins, supplements, herbs, or nicotine) to be used in the study. Indicate whether they have FDA approval, and list any limitations for their use:

No drugs or supplements will be used in the study.

7.3 List all devices, how they will be used, their purpose in the study, and if they will be used in a manner consistent with their approved uses. If they will be used in ways that are not yet FDA approved, indicate whether they need an IDE or a determination that they are exempt from the IDE Determination. If a determination of significant risk or non-significant risk is needed for any of the devices, include the researcher's recommendation for each of those devices:

Magnetic Resonance Imaging: Participants will receive an MRI scan. The Siemens 3T scanner has been approved by the FDA and will be used in a manner consistent with approval.

Metabolic Chamber: Participants will undergo metabolic measurements in a metabolic chamber in a manner consistent with approved uses.

7.4 If the drug is investigational (has an IND) or the device has an IDE or a claim of abbreviated IDE (non-significant risk device), include the following information:

- *Identify the holder of the IND/IDE/abbreviated IDE.*
- *Explain procedures followed to comply with sponsor requirements for FDA regulated research for the following:*

	Applicable to:		
FDA Regulation	***IND Studies***	***IDE studies***	***Abbreviated IDE studies***
21 CFR 11	***X***	***X***	
21 CFR 54	***X***	***X***	
21 CFR 210	***X***		
21 CFR 211	***X***		
21 CFR 312	***X***		
21 CFR 812		***X***	***X***
21 CFR 820		***X***	

N/A

8.0 Procedures Involved

8.1 Describe and explain the study design:

This is a within-subject analysis plan where participants receive at least 3 beverages and undergo blood draw, indirect calorimetry measurements, and an MRI scan.

8.2 Provide a description of:

- *All research procedures being performed*
- *If the study has more than one procedure, session, and/or subject population, describe each procedure, session, and/or study population separately. For complex studies, you are encouraged to include a figure or chart.*

PILOT STUDY:

Overview

The sugars and non-nutritive sweeteners will be using in our full study all have different levels of sweetness due to their chemical structures. To decide what amounts of each of these to use, we will conduct a) pilot study, in which a convenience sample of participants (~20) will taste and rate a variety of mixtures of sugars and non-nutritive sweeteners. To reduce the potential for "tasting fatigue," only a limited number of mixtures will be tasted during a single 2-hour session, but participants will be allowed to complete as many pilot study sessions as they choose.

Recruitment

A convenience sample of participants will be recruited primarily via word-of-mouth or email from our lab's Ripple database (see section 9.4 for information about Ripple). Emails will be generated for individuals who opted-in to contact about participation in future studies with our lab when they filled out a screening survey. See supporting documents for email wording.

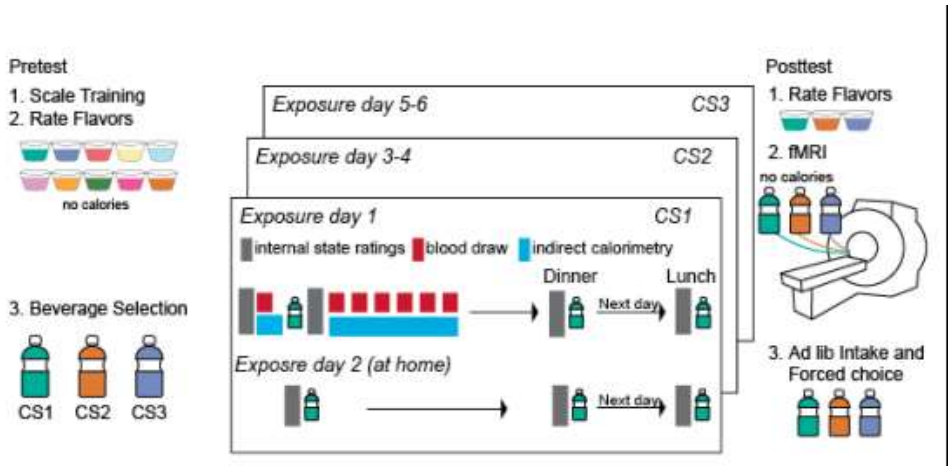
Informed Consent

Participants will be sent via email the consent form for the study at least 24 hours in advance of their scheduled consent session. Upon arrival for the session, participants will have ample time to review and sign the consent form prior to beginning the training session. Research personnel will be available to answer any questions the participant may have.

Flavor and Sweetness Ratings

Participants will be trained on how to use standard perceptual rating scales used in the study. Then, they will taste, rate, and rank each mixture using these scales. All mixtures will be within common sweetness concentrations found in commercially available beverages (i.e., we will not provide sugar or non-nutritive sweetener amounts that would commonly cause gastrointestinal discomfort in most people). Data from this pilot study will then be used to determine acceptable concentrations of sugars and non-nutritive sweeteners to be used in the full study.

FULL STUDY:



OVERVIEW:
Approximately 200 participants will be recruited from the general public for this randomized crossover study conducted at the FBRI. Our goal is to have approximately 140 participants complete the study. The duration of participation will be no more than 6 weeks for each aim. The figure above depicts the overall study design and procedures for both Aims 1 and 2. The study design is the exact same for Aims 1 and 2, but the beverages selected for conditions "CS1, CS2, and CS3" are different, depending on aim. For Aim 1, conditions will include fructose, glucose, and sucrose. For Aim 2, conditions will include sucrose, high fructose corn syrup (HFCS), and non-nutritive sweetener+sucrose. Each CS drink will be consumed over a 1-week period in sequential weeks without "washout" weeks in between.

Recruitment and Screening:
Approved study recruitment materials will be distributed in the follow ways. Flyers will be posted on bulletin boards and approved posting sites throughout Roanoke, Blacksburg, and surrounding areas. Digital advertisements will be displayed on DiFeliceantonio lab and CHBR social media, VT News, and in list serves with permission. All advertisements will include a link to the lab website where there will be approved language describing the research study. Interested participants will fill out a general screening survey. All screening surveys will be delivered through Ripple (a HIPAA-compliant, secure database software; see section 9.4 for description). This screening survey will be used to determine study eligibility. Once the general screening survey has been completed, participants who may be eligible for this study will be contacted via email (see attachment 'Screening email'). If participants choose to complete the study-specific screening form and are eligible for this study, they will be contacted to schedule a consent session.

CONSENT SESSION (Called "pretest" in study design figure above) (~2.5 hours):
Informed Consent
Participants will be sent via email the consent form for the study at least 24 hours in advance of their scheduled consent session. Upon arrival for the session, participants will have ample time to review and sign the consent form prior to beginning the training session. Research personnel will be available to answer any questions the participant may have.

Anthropometrics and Clinical Measures
Participants' height, weight, hip circumference, and waist circumferences will be measured. Height will be measured using a stadiometer, weight using a body weight scale, and waist and hip circumference using a standard Gulick tape measure. A fingerstick blood sample will be collected for point-of-care hemoglobin A1C assessment to ensure eligibility. A lancing device and single-use lancet will be used to obtain a finger stick. A point-of-care A1C analyzer and appropriate cartridges will be used to assess hemoglobin A1C.

Flavor Ratings
The participants will first be trained by the researcher on how to use the standard perceptual rating scales. Then participants will taste and make ratings of beverages using the standard rating scales. After the participant has completed the tasting protocol, the researcher will determine three flavors to be used in the beverages the participants will consume during the behavioral conditioning sessions.

Mock MRI Training
Participants will also complete a "mock" MRI training, in which they learn to swallow small amounts of liquids while lying supine. (Note: most people have no difficulty learning to swallow small quantities of liquid while lying down). This training will be performed in an MRI simulator that is the same bore size and similar environment to the real scanner, but does not contain a magnetic field. Participants who have difficulty with supine swallowing or who express discomfort in the simulated fMRI environment may be excluded from the study or only undergo portions of the study that do not involve fMRI scanning. If not completed during the consent session, participants will be asked to complete this training as part of another conditioning session. Compensation for this "mock" scan is built into the compensation for the consent session.

Dietary Intake
Participants will be instructed to consume the same breakfast meal the morning of each conditioning session and lunch beverage exposure (described below, see In-Person Conditioning Sessions and At-Home Conditioning Sessions). The breakfast meal will be approximately 20% of their estimated total daily energy needs and composed of ~15% of calories from protein, 30% from fat, and 55% from carbohydrate. Estimated energy needs will be calculated using a validated equation that uses the participant's height, weight, sex, and age. Participants will work with research personnel to develop the appropriate meal choice to meet the specified nutrient composition and fit their individual preferences. Participants will be responsible for preparing these meals at home. They will be instructed to consume only plain water after finishing this meal until their scheduled conditioning session.

Dietary, Physical Activity, and Health Behavior Questionnaires
After the training session, participants will receive an email containing links to dietary-physical activity- and behavior-related questionnaires in REDCap to complete. It is estimated that it will take the participant approximately 45 minutes to complete all of the

questionnaires. These questionnaires will be sent using the automated invitation function in REDCap, which will generate an email containing unique links for each survey for each participant. Only participant email addresses will be stored as participant identifiers to track survey responses for data collection.

IN-PERSON CONDITIONING SESSIONS (~4 hours each, ~12 hours total):
These sessions will occur in sequential weeks with at least 3 days in between in-person sessions.
Fast and Refrain from Exercise
Participants will be asked to refrain from exercise for at least 24 hours and fast for at least 3 hours prior to each conditioning session.

Consumption of Planned Breakfast Meal
Participants will be instructed to consume the same breakfast meal the morning of each conditioning session. They will have planned this meal with research personnel during the consent session. They will be responsible for procuring and/or preparing these meals at home. They will be instructed to consume only plain water after finishing this meal until their scheduled conditioning session or take-home lunch beverage consumption time.

Internal State Ratings
Participants will rate their hunger, thirst, and fullness on standard visual analog scales while fasted and after consumption of the beverage, along with perceptual ratings about the beverages consumed during the session.

IV Blood Draws
An IV catheter will be inserted into the participant's arm for venous blood draws throughout the session. This catheter will remain in the participant's arm for the duration of the session (~4 hours). A fasting baseline draw will be made, and then six additional blood draws after the participant consumes the conditioning beverage will be conducted throughout the remaining session. Two tubes of no more than 5 ml each will be collected at each blood draw (14 tubes total). This is approximately 126 ml of blood in total (~8-9 tablespoons). This is roughly equivalent to 1/4 the amount of blood given during a typical blood donation. All blood draws will occur while the participant is reclining on a bed inside a metabolic chamber (see Metabolic Chamber Indirect Calorimetry below). For the draws, the researcher performing the blood draws will reach their arms into the chamber via ports on the side of the chamber walls. Participants will be able to communicate with research personnel using a 2-way intercom system, and the walls of the metabolic chamber are clear plexiglass that allow researchers and participants to see each other throughout the measurement. IV blood draws occur concurrently with metabolic chamber measurements (see below). The IV catheter line will be flushed with normal saline between each blood draw to prevent coagulation in the catheter tubing. After the final blood draw, the catheter will be removed and the venipuncture site secured with sterile tape or bandage. Should the IV catheter placement fail or become unusable during the session, the catheter will be removed and participants will continue with the metabolic chamber indirect calorimetry portion of the session.

Metabolic Chamber Indirect Calorimetry
Participants will rest reclined on a bed inside the metabolic chamber for the duration of the measurement. The chamber is an open-circuit system in which fresh air is pumped into the room at a specified flow rate. Metabolic chamber measurements involve non-invasive collection and measurement of oxygen and carbon dioxide concentrations expired through respiration. Metabolic chamber measurements occur concurrently with IV blood draws (see above). Participants will be able to communicate with research personnel using a 2-way intercom system, and the walls of the metabolic chamber are clear plexiglass that allow researchers and participants to see each other throughout the measurement. A fasting measurement will be collected for approximately 30-60 minutes; during this time a fasting blood draw will also be collected (see IV Blood Draws above). After the fasting measurement, participants will consume the conditioning beverage, and a post-beverage measurement will be collected for approximately 3 hours.

Beverage Consumption
Participants will consume the single 355 ml, 110 kcal conditioning beverage within 5 minutes. The conditioning beverage contains flavoring, citric acid (commonly used in sugar-sweetened beverages [e.g., Gatorade]), deionized water, and the sugar or sugar + non-nutritive sweetener stimuli being tested. Participants will rate their hunger, thirst, and fullness on standard visual analog scales after consumption of the beverage, along with perceptual ratings about the beverages consumed during the session.

Take-Home Beverages
At the end of the session, participants will be provided 5 bottles of the same conditioning beverage to be consumed at home. One bottle will be used for the at-home conditioning session (see below), and the remaining 4 total bottles will be consumed at the following times: 1 hour before dinner the same day as the in-person and at-home conditioning sessions (1 bottle for each conditioning session), and 1 hour before lunch the day following the in-person and at-home conditioning session (1 bottle for each session).

AT-HOME CONDITIONING SESSIONS (~15 minutes each, 45 minutes total):
Fast and Refrain from Exercise
Participants will be asked to refrain from exercise for at least 24 hours and fast for at least 3 hours prior to each conditioning session.

Consumption of Planned Breakfast Meal
Participants will be instructed to consume the same breakfast meal the morning of each at-home conditioning session. They will have planned this meal with research personnel during the consent session. They will be responsible for procuring and/or preparing these meals at home. They will be instructed to consume only plain water after finishing this meal until their scheduled conditioning session time.

Zoom Monitoring of Consumption
A Zoom link will be sent to the participant prior to the scheduled at-home conditioning session time. During the session, participants will turn their camera on to allow the

researcher to watch them drink the conditioning beverage. This session will NOT be recorded. At the end of the session, participants will be reminded to consume the take-home beverages at the appropriate specified times: 1 hour before dinner the same day, and 1 hour before lunch the following day. Participants will be sent an automated email with a link to a REDCap survey. Participants will rate their hunger, thirst, and fullness on standard visual analog scales before and after consumption of the beverage, along with perceptual ratings about the beverages consumed during the session on this survey.

TAKE-HOME BEVERAGE CONSUMPTION (~5 minutes each, 60 minutes total):
Consumption of Planned Breakfast Meal
Participants will be instructed to consume the same breakfast meal the morning of each lunch beverage consumption time. They will have planned this meal with research personnel during the consent session. They will be responsible for procuring and/or preparing these meals at home. They will be instructed to consume only plain water after finishing this meal until their scheduled lunch beverage consumption time.

Consumption of Take-Home Beverages
Participants will be instructed to consume the take-home beverages 1 hour before dinner the same day as their conditioning session (either in-person or at-home; see above) and 1 hour before lunch the day following their conditioning session (either in-person or at-home). Participants will be sent surveys to verify beverage consumption times and amounts via REDCap. These surveys will be sent using the automated invitation function in REDCap, which will generate an email containing unique links for each survey for each participant. Only participant email addresses will be stored as participant identifiers to track survey responses for data collection.

POST-TEST SESSION (~3 hours):
Fast and Refrain from Exercise
Participants will be asked to refrain from exercise for at least 24 hours and fast for 3 hours prior to each conditioning session.

Internal State Ratings
Participants will rate their hunger, thirst, and fullness on standard visual analog scales while fasted and periodically throughout the session.

Flavor Ratings
Participants will taste and make ratings of flavored beverages using standard rating scales. These are the same beverages and flavors used throughout the study.

MRI Scan
The MRI scan will occur at the Human Neuroimaging Laboratory at FBRI. MRI safety will be reviewed with the participant by an FBRI-approved scanner operator prior to entering the scanner room containing the magnet. Participants and experimenters will remove metal from their bodies and clothing (e.g., jewelry, cell phones, coins, etc.). Participants will also complete an MRI safety form to ensure they qualify to have an MRI performed (included in supporting documents). Prior to entering the scanner room with

the magnet, experimenters will use a metal detection wand to scan over the participant to ensure no metal that could be subject to the magnet's pull is detected (e.g., zippers or grommets on clothing cannot be pulled off by the magnet). Inside the scanner room, participants will lie on a table that slides into the bore of the scanner. Using a head coil, functional images will be acquired using an EPI bold sequence. Participants will wear disposable earplugs and other ear protection to minimize the amount of noise heard during the scan (magnetic field gradient changes during the scan can be loud). Participants are able to communicate with the experimenter via either a speaker and microphone system (between scanning segments) or with an emergency squeeze ball (during scan acquisition). Participants will be instructed to talk with experimenters between scanning segments via microphone-and-speaker system and to use the squeeze ball during scan acquisition for emergencies (e.g., they begin to feel claustrophobic during the scan). The experimenters will check in with participants periodically between scanning segments to ensure participant comfort.

Scanner Taste Tasks
Participants will taste small amounts of liquid stimuli that will be presented according to a standard paradigm we have successfully used before in taste MRI. In brief, hydraulic pumps will be used to pump solutions through plastic beverage tubing attached to a mouthpiece to the subject lying in the scanner. The mouthpiece will be sterilized and new beverage tubing will be used for each participant. The mouthpiece will be anchored to the head coil so that the end comfortably rests on the anterior tongue, just inside the participant's mouth. This will not interfere with breathing through the mouth, and participants will always be able to breathe through their noses. The solutions will be dispensed through one tube at a time. The overall flow rate of solution into the mouth will be very slow (~0.5ml of a liquid over ~3-5 seconds).

Ad Libitum Intake
Participants will be given access to bottles containing the conditioning beverages used during their study sessions for 30 minutes. They will be allowed to drink as much or as little of the beverages as they would like. Amounts of beverages consumed will be recorded as data.

Forced Choice
Participants will be offered the conditioning beverages used during their study and asked to choose one to take home with them. This is a measure of "forced choice" and will be recorded as data.

8.3 Describe:

- *Procedures or safeguards intended to reduce the probability and magnitude of risks. (For example: Reducing the risk of injury in a virtual reality study either by having the subjects sit during the study or by providing an obstacle-free space for walking.)*
- *Be sure to describe all drugs and devices used in the research, when they will be administered or used, and their purpose.*

- *Methods used to collect data about subjects. Please upload all data collection forms to Protocol Management. Some common examples are:*
  - *Screening questionnaires*
  - *Survey(s), including online surveys*
  - *Demographic questionnaire(s)*
  - *Interview guide(s), e.g., questions or pool of questions for semi-structured interviews*
  - *Focus group guide(s)*
  - *Other documents used to collect data*

Procedures or safeguards intended to reduce the probability and magnitude of risks: MRI. Every effort will be made to ensure the subject's comfort and to reduce any minimal risk. First and foremost, subjects will be thoroughly screened to make sure that MRI contraindications are not present. If, at any time, subjects experience discomfort, dizziness, or claustrophobia during the scan, they will have access to an emergency squeeze bulb which they can squeeze to get the attention of the experimenters. Experimenters will then check in with the subject, and if requested, subjects will be immediately removed from the MRI machine. Additionally, the staff operating the MRI will have completed Advanced MRI/Operator Training which is required for those persons wishing to conduct research on the Human Neuroimaging Lab (HNL) MRIs. Training consists of a safety training, full-day advanced user training, scanning observation, and hands-on practice, supervised scanning. Topics include safety and emergency procedures, subject preparation and screening, scanner set-up and operation, and troubleshooting. Each person must also complete at least 4 hours of observation (to include 2 different studies) and at least 16 hours of supervised scanning (to include 4 different studies). Certification is granted upon completion of the training and passing of a competency exam.

An MRI Safety Screening Form will Be completed by each participant and reviewed by trained staff in order to ensure there are no contraindications to MRI scanning.

Venous Blood Draw. The potential risks associated with the blood draw procedure include hematoma from failure to use proper blood drawing techniques and infection if proper infection and control practices are not observed and used (WHO). These may be associated with some discomfort, but present very little danger to subjects' welfare. To reduce this risk, all blood draws will be performed by a trained and skilled medical technologist, phlebotomist, or nurse in the appropriate laboratory setting. In addition, a maximum of three attempts will be made to obtain blood during each individual blood draw. Rarely, a blood clot will form or infection, inflammation, or bleeding can occur at the site. If inflammation at the site does occur, we will apply a warm compress to the site and elevate the arm to reduce it.

Fingerstick Blood Draw. The potential risks associated with performing a fingerstick blood draw are minimal. The likely risks involve pain and bleeding in the fingertip at the collection site. Rarely, an infection may occur at the puncture site. To minimize these risks, participants and study staff will be trained on the proper technique for performing a capillary blood sample and how to care for the site after the sample is obtained.

Catheter Blood Draw: Some pain or discomfort may be experienced when the catheter is inserted in the vein, but this should persist for only a short time. During the blood draws, there may be pain and/or bruising at the site where the IV catheter is inserted. In about 1 in 10 or 10% of the cases, a small amount of bleeding under the skin will cause bruising. The risk of a blood clot forming in the vein is about 1 in 200, while the risk of infection or significant blood loss is 1 in 1000. There is a small risk of the vein becoming inflamed and/or painful in the hours or days after the needle is removed. To reduce this risk, all catheter placements and blood draws will be performed by a trained and skilled medical technologist, phlebotomist, or nurse. In addition, a maximum of three attempts will be made to insert the IV catheter at different sites. If the participant feels faint during or after a blood draw, research personnel will immediately have the participant lie back on the bed inside the metabolic chamber.

Metabolic Chamber Indirect Calorimetry: There are no known risks associated with the method of indirect calorimetry. However, some people may feel uncomfortable or anxious while inside the metabolic chamber. Participants will be able to breathe normally during the entirety of the testing procedure. They will also be able to see and communicate with the experimenter and will be able to tell the experimenter if they would like to terminate the measurement.

8.4 What data will you collect during the study and how you will obtain them? Please include descriptions of electronic data collection, database matching, and app-based data collection:

Ripple (see section 9.4 for Ripple description) will be used to collect screening information. Information about the beverages participants will be consuming at home as well as dietary, physical activity, and behavior information will be collected through REDCap. MRI safety information will be collected via paper form (included in supporting documents). Perceptual ratings will be collected using a laptop or tablet and custom scripts written in R or Python (PsychoPy). Blood glucose will be measured using a Hemocue point-of-care system, and insulin will be measured using the Immunolite 1000 Immunoassay System or ELISA. Other blood metabolites will be measured from stored samples using ELISAs or other appropriate wet lab equipment and techniques. Brain images will be acquired during fMRI scans. Metabolic measurements will be collected using a metabolic chamber from MEI Research, Ltd. All data collected during the study will be stored on FBRI's instance of REDcap and/or FBRI's secured server.

8.5 Who will transcribe or code audio and/or video recordings?:

There will be no audio or video recordings.

8.6 Include a description of any deception to be used in the study. Include justification for the use of deception (why the deception is necessary), describe the debriefing process, and describe how the study meets <u>all</u> the following criteria for alteration of consent (deception is considered an alteration of informed consent):

- *The research involves no more than minimal risk to the subjects*
- *The alteration will not adversely affect the rights and welfare of the subjects*
- *The research could not practicably be carried out without the alteration/deception*
- *(Optional but encouraged in most cases) Subjects will be provided with additional pertinent information after participation (i.e., debriefing for studies involving deception)*

There will be no deception.

8.7 If the study involves long-term follow-up (once all research related procedures are complete), describe what data will be collected during the follow up period and when it will occur:

There are no long-term follow-ups.

9.0 Data and Specimen Long Term Storage and Use

9.1 If you will store data or specimens for future use, describe where you will store the data or specimens, how long they will be stored, and how and by whom the data or specimens will be accessed:

Indirect calorimetry data will be stored on a secure network drive provided by FBRI. Behavioral (e.g., last time food eaten before measurement, last bout of exercise before measurement, etc.), anthropometric, responses to questionnaires, and pertinent metabolic variables will be stored in FBRI's

instance of REDCap. Data will be stored indefinitely for future analysis and use in developing future protocols. Only individuals with FBRI access to secure network drives and FBRI's instance of REDCap will have access to stored data. Plasma and/or serum aliquot samples will be frozen and stored for future analysis of insulin and other metabolites. Additional aliquots of plasma and/or serum will be stored indefinitely after the completion of data analysis to allow for replicate testing to ensure data quality and for additional analyses as the field moves forward.

9.2 For specimens, list the data to be stored or associated with each specimen:

Serum and plasma blood samples for storage will be labeled with the participant's 4-digit ID number, date collected, and conditioning beverage associated with that sample.

9.3 Describe the procedures to release data or specimens outside of the research team, including the process to request a release, approvals required for release, who can obtain data or specimens, and what data will be provided with specimens:

No specimens will be released.

9.4 Describe the identifiers to be included with stored data or specimens, as well as any key or code that could be used to make them identifiable. Describe where the code will be stored, who will have access to it, and when it will be destroyed:

Data will be coded using a 4-digit sequentially-generated ID number unique to each participant. The key linking the participant name or identifiable information and the ID number will be stored in Ripple.

Ripple is a secure web application designed for storing and managing personal identifiable information of research participants. Ripple was initially developed at the University of Michigan to provide a user-friendly, web-based secure interface where research teams can centralize the storage and management of research participants' personal information. Information stored in Ripple is kept in fully encrypted format inside dedicated databases that are segregated from other Ripple accounts and thus only authorized study staff will have access to the study data. Likewise, Ripple infrastructure complies with the privacy and security guidelines of the Health Insurance Portability and Accountability Act

(HIPAA), including 2048-bit data encryption in transit and at rest, automatic logoff, audit trail, daily backups in triplicate dedicated servers, firewall, custom access permission for lab members, zxcvbn password strength estimation, and enterprise administrative safeguards to prevent unauthorized staff from accessing participant information. Only personnel who are direct members of the research team will have access to personally identifiable information stored in Ripple. The link between the coded designation and personal information will only exist on this secure platform.

All study data will be encrypted and kept on either a server or FBRI's instance of REDCap, both managed by FBRI's Information Technology (IT). Only authorized research team members, who have authorized FBRI IT-assigned credentials, will be able to access the network drive server and REDCap.

Any paper documents, such as signed consent forms and MRI safety forms, will be stored in a locking filing cabinet accessible only by authorized research team members.

Per Virginia Tech policy in order to compensate participants, social security number (or Taxpayer ID) and mailing address will be collected via a W-9 or W-8BEN form. The participant address and SSN will be entered into the ClinCard system, which is a HIPAA-compliant payment system approved by Virginia Tech. After entry, this form will be given to and stored by the central administration at the FBRI to be used if it is required that a tax statement be sent to the participant, based on Federal Income Tax regulations. Study staff will not store documents with SSN or address used for the purpose of payment.

Identifiable information will be destroyed at the written request of the participant.

9.5 Please select the identifiers you will obtain (whether directly from participants or from another source), including but not limited to:

☒	*Name*
☒	*Geographical subdivisions smaller than a state, including street address, city, county, precinct, zip code, and equivalent geocodes (note, the initial three digits of a zip code are not considered identifiable)*
☒	*Elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death, and single year of age over 89 and all elements of dates (including year) indicative of such age (note, such ages and elements may be aggregated into a single category of age 90+)*

☒	*Phone numbers*
☐	*Fax numbers*
☒	*Electronic mail addresses (e-mail)*
☒	*Social Security numbers*
☐	*Medical record numbers*
☐	*Health plan beneficiary numbers*
☐	*Account numbers*
☐	*Certificate/license numbers*
☐	*Vehicle identifiers and serial numbers, including license plate numbers*
☐	*Device identifiers and serial numbers*
☐	*Web Universal Resource Locators (URLs)*
☐	*Internet protocol (IP) address numbers*
☐	*Biometric identifiers, including finger and voice prints (audio recording)*
☐	*Full face photographic images and any comparable images (including video recording)*
☐	*Student record number or identification number*
☐	*User name for online or computer accounts*
☐	*Any other unique identifying number, characteristic, or code (note this does not mean the unique code assigned by the investigator to code the data):* Click here to explain.

10.0 Sharing of Results with Subjects

10.1 Describe whether you will share results (study results or individual subject results, such as results of investigational diagnostic tests, genetic tests, or incidental findings) with subjects or others (e.g., the subject's primary care physician). If so, describe how you will share the results and include this information as part of the consent document. Upload materials you will use to explain the results to subjects:

Participants will be given the option to receive their clinical lab results collected during the study, including fasting blood glucose value, hemoglobin A1C value, resting metabolic rate, BMI, and waist-to-hip ratio. Along with these individual results, participants will receive general health information about clinical 'norms' and general interpretation of values; research personnel will not provide specific health interpretation of individual results. Participants will be directed to contact their primary care physician or healthcare provider if they have specific questions regarding interpretation of their individual results. See the supporting documents for participant individual result documents and materials. Participants will also be given the option to receive a picture of their brain, taken during the MRI scan. If there are incidental findings potentially consistent with an undiagnosed condition (e.g., hemoglobin A1C measured >6.5%),

participants will be encouraged to follow-up with their primary care physician for clinical evaluation and testing.

11.0 Study Timelines

11.1 Describe:

- *The duration of an individual subject's participation in the study (for example, 1 hour, 2-4 weeks, 3-5 years).*
- *The amount of time expected to enroll all study subjects (weeks, months, years, etc.)*
- *The amount of time expected for the investigators to complete this study including primary data analyses.*

Pilot Study: ~2 hours per session (participants can choose how many sessions they would like to complete)

Full Study: ~19 hours total (over a ~5-week period)

Consent Session: ~2.5 hours

In-Person Conditioning Sessions: ~4 hours each, 12 hours total

At-Home Conditioning Sessions: ~15 minutes each, 45 minutes total

Take-Home Beverage Consumption: ~5 minutes each, 1 hour total

Post-Test Session: ~3 hours

The study, including all recruitment and data analysis, is expected to last 6 years.

12.0 Inclusion and Exclusion Criteria

12.1 Describe how you will screen individuals for eligibility. When will screening occur and what procedures will you use? Upload any screening scripts or surveys to Protocol Management:

Screening surveys will be delivered through Ripple (see information about Ripple in section 9.4) via web address links and QR codes posted on flyers and social media. Individuals interested in a study will complete 2 surveys; one serves as a first-round general survey screening for exclusion criteria pertinent to all our lab's studies, and the second contains questions to screen eligibility for this study specifically. If individuals choose to

complete the study-specific screening form and are eligible for this study, they will be contacted to schedule a consent session. Individuals may also be contacted via email with follow-up questions to ensure eligibility. If follow-up questions involve potentially sensitive information, a phone call will be used rather than an email. Once enrolled in the study, participants will also complete an MRI safety screening form to ensure they are eligible to complete the MRI.

12.2 Describe the eligibility criteria that define who will be included and who will be excluded from enrollment for each procedure of your study. Include any geographic criteria (e.g., Virginia Tech undergraduate students, a national sample of adults with engineering degrees, minors aged 8-12 in the New River Valley, university faculty in Virginia and Paris, France):

Inclusion Criteria:
1. Age 18-45 years
2. BMI between 18.5-27 kg/m2
3. Not pregnant or planning to become pregnant during study participation
4. Residing in the Roanoke area and/or willing/able to attend sessions at FBRI
5. Weigh at least 110 lbs

Exclusion Criteria:
1. Current inhaled nicotine use
2. History of alcohol dependence.
3. Current or past diagnosis of cardiometabolic disease or problems, including diabetes, endocrine, heart, or thyroid problems, that may influence study outcomes
4. Hemoglobin A1C >5.7%
5. Taking medications known to influence study measures (including ADHD, allergy, antidepressant, antipsychotic, anxiolytic, , blood pressure, blood glucose, cholesterol, thyroid, sleep, or weight loss medications)
6. Active medical or neurologic disorder, including cardiometabolic conditions or gastrointestional conditions that may influence study outcomes
7. Recent change in body weight (gain or loss of > 5 lbs within the past 3 months)
8. Current shift work (typical pattern of work/activity overnight)
9. Previous weight loss surgery
10. Adherence to a special diet within the past 3 months (e.g., low-carb or ketogenic diet, exclusion of food groups/specific macronutrients, intermittent fasting, etc.)
11. Allergy to any food or ingredient included in the study diets, meals, or beverages
12. Currently pregnant or planning to become pregnant during study participation
13. Claustrophobia
14. Contraindications for MRI, including pacemaker, aneurysm clips, neurostimulators, cochlear or other implants, metal in eyes, regular work with

steel, etc. (Note: This is an fMRI-specific exclusion criterion. Participants may be allowed to participate in all other study sessions and measures that do not involve fMRI.)
16. Use of substances (or combinations of substances) in doses and frequencies that could influence neural outcomes of study.

12.3 Indicate specifically whether you will include or exclude each of the following special populations: (You may not include members of these populations as subjects in your research unless you indicate them in the description of your subject population.)

- *Minors, as defined by state law where the study is performed (infants, children, teenagers)*
- *Pregnant women (can be included in minimal risk studies by mentioning in section 13.1)*
- *Prisoners (including all incarcerated individuals)*
- *Adults not capable to consent on their own behalf*

We will exclude minors, prisoners, pregnant women and adults not able to consent on their own behalf.

13.0 Vulnerable Populations

13.1 If the research involves individuals who are vulnerable to coercion or undue influence, please describe additional safeguards you will include to protect their rights and welfare. Consider the applicable items listed below:

- *If the research involves Virginia Tech students, indicate whether these are students of any of the investigators. If so, describe whether the activities will take place during class time as part of the curriculum and the steps you will take to reduce the possibility that students feel obliged to participate in order to improve their course grade. The HRPP can provide further guidance as needed. Describe*

whether you will request access to student records (e.g., SAT, GPA, GRE scores).

- *If the research involves employees of Virginia Tech or the research sponsor, describe steps you will take to ensure that the employees are freely participating and describe how their data will be protected from inspection by their supervisors.*
- *If the research involves Virginia Tech NCAA athletes, you must obtain approval from the athletic department.*
- *For research involving Montgomery County Public Schools, you must obtain county approval (after obtaining contingent Virginia Tech approval). Other locales have different requirements; please check on these and describe here. Approval is typically granted by the superintendent, principal, and classroom teacher (in that order). Approval by an individual teacher is insufficient. School approval, in the form of a letter or a memorandum should be uploaded as a supporting document.*
- *If the research involves pregnant women, review "CHECKLIST: Pregnant Women (HRP-412)" to ensure that you have provided sufficient information in this protocol.*
- *If the research involves prisoners, review "CHECKLIST: Prisoners (HRP-415)" to ensure that you have provided sufficient information in this protocol.*
- *If the research involves persons who have not attained the legal age for consent to treatments or procedures involved in the research (minors), review the "CHECKLIST: Minors (HRP-416)" to ensure that you have provided sufficient information in this protocol.*
- *If the research involves cognitively impaired adults, review "CHECKLIST: Cognitively Impaired Adults (HRP-417)" to ensure that you have provided sufficient information in this protocol.*

All subjects will be informed of the purpose of the study, the potential value of the study to society, the lack of value to the subject personally, and all potential risks to the subject. Study staff will explain to the participants that they are under no obligation whatsoever to participate, and that if they wish to discontinue their involvement at any time during the study, they are free to do so without penalty. Employees will be told that their employment statuses and evaluations will in no way be affected by their decision to participate or not participate in the study. Students will be advised that their decisions to participate will not affect their student statuses, grades, coursework, or extra-curricular activities.

14.0 Number of Subjects

14.1 Indicate the total number of subjects to be enrolled and how this number was determined (e.g., sample size calculation [show], number of available subjects in a finite pool, number of tests funding award would allow):

For the pilot study, we plan to consent 25 participants with the intent of having 20 complete the pilot study session.

For the full study, we plan to consent approximately 200 participants with the intent of having 70 participants complete Aim 1 and 70 participants complete Aim 2. These numbers were determined using power calculations based on other studies with similar designs conducted by our lab. We anticipate ~30% rate of either drop-out or exclusion after enrollment (e.g., unable to determine flavors equally liked and unfamiliar for the conditioning paradigm, hemoglobin A1C outside of the inclusion range, scheduling challenges, etc.).

14.2 If this is a multi-site study, indicate the number of subjects to be enrolled at this site and the total to be enrolled from all sites:

Single site

14.3 If applicable, indicate the number of potential subjects you expect to screen for enrollment, and the number of subjects you will need to complete the research procedures:

For the pilot study, we anticipate screening via the online screening process approximately 25 potential participants. We anticipate enrolling and screening approximately 20 participants during the Consent Session. We intend to have 20 participants complete the pilot study.

For the full study, we anticipate screening via the online screening process approximately 2000 potential participants. We anticipate enrolling and screening approximately 200 participants during the Consent Session, We intend to have 70 participants complete Aim 1 and 70 participants complete Aim 2. We anticipate that a majority of these participants will complete only 1 aim; however, it is possible and allowable for participants to complete both aims if they qualify for both (i.e., at least 6 flavors are rated as equally liked and equally unfamiliar in the Consent Session).

14.4 If the study has more than one procedure, indicate the total number of subjects to undergo each procedure separately:

Pilot Study (single session for each sweet mixture): 20 participants

Full-Study

Consent Session (flavor tasting/rating, anthropometrics, fingerstick sample for HbA1C): 200 participants

In-Person Conditioning Session (IV catheter blood draws, metabolic chamber indirect calorimetry): Aim 1: 70 participants; Aim 2: 70 participants

At-Home Conditioning Session (drink beverages on Zoom): Aim 1: 70 participants; Aim 2: 70 participants

Take-Home Beverage Consumption (drink beverages and complete surveys): Aim 1: 70 participants; Aim 2: 70 participants

Post-Test (flavor tasting/rating, MRI scan): Aim 1: 70 participants; Aim 2: 70 participants

15.0 Recruitment Methods

15.1 Describe when, where, and how you will recruit potential subjects:

Approved study recruitment materials will be distributed in the follow ways. Flyers will be posted on bulletin boards and approved posting sites throughout Roanoke, Blacksburg, and surrounding areas. Digital advertisements will be displayed on social media, VT News, and in listserves with permission.

All advertisements will include a link to the lab website where there will be approved language describing the research study. Interested participants will fill out a general screening survey. All screening surveys will be delivered through Ripple. This screening survey will be used to determine study eligibility. Once the general screening survey has been completed, participants who may be eligible for this study will be contacted via email (see attachment 'Screening email'). If participants choose to complete the study-specific screening form and are eligible for this study, they will be contacted to schedule a consent session.

Opt-In Database
Individuals who complete the screening survey in response to other studies in the lab will have the opportunity to opt-in to being notified of future studies our lab is conducting. One question on the screening survey asks if individuals would like to be contacted if they potentially qualify for any future studies. Individuals who have opted-in to being notified of future studies receive an email containing information about the study and an email address for contacting the research study team. The email will be sent through Ripple, the secure web application we are using for storage and management of personally identifying information of research participants. Individuals who are interested in scheduling a consent session will undergo a brief screening to ensure they have had no

changes to their health history or other screening survey questions since they first completed the survey that would preclude them from participating.

Carilion Clinic Recruitment

Flyers will be posted throughout Carilion Clinic waiting rooms and offices. Physicians and other providers will also be notified of this study and asked to encourage individuals who may qualify to complete the screening survey.

15.2 Describe the source of subjects (for example, clinic patients with specific conditions, students in the library, community members at a gathering, or members of a local gym):

Participants will be recruited from the general public.

15.3 Describe the methods that you will use to identify potential subjects:

Responses on the online screening form will be used to identify potential subjects.

15.4 Describe materials that you will be use to recruit subjects. Attach copies of these documents with this protocol in Protocol Management and be sure to include the IRB protocol number on each document.

- *For flyers, attach the final copy of printed flyers.*
- *For Virginia Tech News, Facebook postings and ads, newspaper ads, websites, MTurk/SONA/online survey systems, etc., attach the final wording and graphics to be used.*
- *For email recruitments, please include the subject line.*
- *For advertisements meant for audio broadcast, please submit the wording of the advertisement prior to taping (to avoid having to re-record with approved language) and submit the final recorded version for IRB review before use.*
- *Describe any compensation to subjects. Separate compensation into appropriate categories, such as: reimbursement for expenses, time and effort, and additional incentives for study participation. For each category, specify the amount (including any pro-rated amount), schedule, and method of payment.*

See above in 15.3 for a list of recruitment materials and methods that will be used.

Pilot Study (~2 hours) - $20

FULL STUDY

Consent and Training Session (~2.5 hrs) - $40
Conditioning Sessions (+Take-Home Beverages) (~4.5 hours each week, 3 weeks total) - $90 each week; up to $270 total
Post-Test Session (~3 hours) - $60
Full Study Completion Bonus - $50

Total for each Aim - $420
Total for both Aims - $800
The remuneration amounts described for sessions in the Full Study above pertain to both Aim 1 and Aim 2. If a participant completes both Aims, they will still only complete 1 Consent Session. In other words, if a participant completes all of Aim 1 and all of Aim 2, they will complete only 1 Consent Session and be paid $800.

Participants will be paid via ClinCard, a prepaid Mastercard that we will provide them at the end of the first session. Participants will be compensated at the end of each session week using the ClinCard system.

16.0 Withdrawal of Subjects

16.1 Describe circumstances under which you anticipate subjects could be withdrawn from the research without their consent:

As is standard with MRI research, if subjects experience unusual sensations, they will be withdrawn. In some cases, due to the magnetic field (as described in risks below) subjects may experience peripheral nerve stimulation, e.g. tingling or twitching. They will be withdrawn from the study if this occurs.

Other potential reasons for withdrawal without participant consent include information on the screening questionnaire that would make participating unsafe for the participant, failure to follow the study protocol as instructed (e.g., not fasting for measurements, failure to consume beverages at home at the appropriate times, consuming foods or beverages outside of those provided by the study on controlled feeding days, etc).

16.2 If applicable, describe any procedures for orderly termination (e.g., discontinuation of a study drug or debriefing after a behavioral intervention):

There are no study drugs or behavioral interventions.

16.3 Describe procedures that you will follow when subjects withdraw from the research, including partial withdrawal from procedures with continued data collection (e.g., participant declines to continue with regular blood draws, but continues with periodic behavioral questionnaires):

The following steps will be taken in the event a participant withdraws from the study and informs us they want their information destroyed:
1. The participant database will be updated that the participant withdrew (for all withdrawals).
2. Participant will be compensated for their time and sessions completed up to time of withdrawal.
3. If the participant requests, all participant identifying information and data collected up to time of withdrawal will be removed from study database(s).
4. If the participant requests, all stored blood samples collected on the participant will be removed from the storage freezer and discarded.
5. If the participant requests, all paperwork associated with the participant will be shredded.

17.0 Risks to Subjects

17.1 List the reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related the subjects' participation in the research. Include for the IRB's consideration a description of the probability, magnitude, duration, and reversibility of the risks. Consider physical, psychological, social, legal, privacy, and economic risks. Do not indicate "No risk" or "N/A." Instead, for studies with very low risk (e.g., anonymous online questionnaire on a mundane topic) indicate "The investigators are not aware of any risks from participation in this study." or "No more than risks than are found in everyday life." The example consent form presents a tabular method for risk information, which you can also use here. Common risk types include:

- *Physical (e.g., potential for pain, discomfort, infection)*
- *Psychological (e.g., potential for stress, discomfort, and/or embarrassment)*
- *Social (e.g., potential for discrimination or stigmatization and disruption of personal and family relationships)*
- *Legal (e.g., potential for disclosure of illegal activity, negligence)*
- *Privacy (e.g., potential for personal information being accessed, used, or disclosed without the subjects' knowledge or consent, breach of confidentiality/security)*
- *Economic (e.g., potential for individuals to lose access to economic services, employment, insurability)*

MRI data collection: The risks associated with collecting fMRI are the same as regular MRI. Movement or heating of metallic implants is a potential risk, therefore those with metallic pacemakers, fragments, or implants will be excluded from the study during screening. Some participants may experience claustrophobia in the scanner; any participant experiencing claustrophobia will be removed from the scanner immediately. fMRI scanners also produce loud noises while running which could be harmful to the subject. Participants will be required to wear earplugs and other ear protection during scans to minimize the noise generated by the scanner.
The Siemens 3 T scanner has been approved by the FDA. However, there may be additional risks associated with scanning at 3.0 T compared to the conventional clinical scanners in the 1.5-2.0 T range. These include:
1. Effect of the static field. There is no conclusive evidence for irreversible or hazardous bioeffects to acute, short-term exposures of humans up to 2.0 T (Shellock and Kanal, 1996). Studies have indicated some side-effects at 4.0 T, namely unusual sensations including nausea, vertigo, and metallic taste (Schenck, 1991). However, there is no evidence that this is either irreversible or harmful. If subjects experience unusual sensations, they will be withdrawn.

2. Effect of the gradient field. MRI operates by rapidly changing small additional fields, called gradients. This will induce small electrical currents in any conductor, and thus could theoretically induce mild peripheral nerve stimulation. However, this is not substantially different at higher magnetic fields since the gradients are separate from the main magnet. There is no evidence that the effect of the gradients is any different at 3 T than at 1.5 T. However, if subjects experience peripheral nerve stimulation, e.g. tingling or twitching, they will be withdrawn.

3. Effect of the RF electromagnetic field. The higher magnetic field strength requires that higher RF frequency pulses are used to excite the protons in the subject's brain. The limits of RF energy that can be safely given to humans has been clearly defined by the FDA: a). The exposure to RF energy below the level of concern is an SAR of 0.4 W/kg or less averaged over the body, and 8.0 W/kg or less spatial peak in any 1 g of tissue, and 3.2 W/kg or less average over the head; or b). The exposure to RF energy that is sufficient to produce a core temperature increase of 1 degree C and localized heating to no greater extent than 38 degrees C in the head, 39 degrees C in the trunk, and 40 degrees C in the extremities, except for patients with impaired systemic blood flow and/or perspiration. We will adhere to the recommendations for the head, which is also monitored by a Siemens built-in monitor.

4. Acoustic Noise Levels. Rapid changes in the currents in the gradient coils of the MRI scanner produce significant levels of acoustic noise. The levels of noise range approximately between 65 and 95 dB, but could have higher peaks. Communications with subjects takes place with the standard intercom system provided by the scanner manufacturer.. Foam ear plugs with a Noise Reduction Rating of at least 30 dB will be provided.

Subjects may experience discomfort, such as anxiety during the MRI scanning. Subject comfort will be reassessed and subjects will be allowed to discontinue any session at any time.

The subject may communicate with the experimenter at any time: 1) before, in between, or after a scan via an intercom device between the scanner and control room, and 2) during a scan via a pneumatic squeeze bulb located in the scanner that triggers an alarm in the control room. Subjects who report discomfort and wish to discontinue their participation will be immediately withdrawn from the scanner. Subjects who are withdrawn from the study will be compensated for their participation to that point.

IV Catheter Blood Draws:
All catheter placements and draws will be performed by a trained and skilled medical technologist, phlebotomist, or nurse in the appropriate laboratory setting with proper antiseptic technique. However, there are still some risks of this procedure. Some pain or discomfort may be experienced when the catheter is inserted in the vein, but this should persist for only a short time. During the blood draws, there may be pain and/or bruising at the site where the IV catheter is inserted. In rare cases infection or blood clot may occur at the IV catheter site. These are the same risks associated with IV catheter placement in any medical setting. Inclusion criteria for this study include non-pregnant adults who weigh at least 110 lbs. Blood collection during draws will total ~189 ml over a 3-week period (~63 ml per week), well below the minimal risk cutoff of 550 ml in an 8-week period. In this study, blood collection occurs 1 time per week. Thus, this study fits the defined criteria for imposing minimal risk.

Beverage and food stimuli: All components of the beverage and food stimuli are readily available outside the laboratory (grocery stores, etc.) and are likely already consumed by the participants. There are no known risks associated with these stimuli.

Indirect Calorimetry. There are no known risks associated with the noninvasive indirect calorimetry methods. These non-invasive procedures are simple and painless, and normal clothes can be worn during the procedures. For the metabolic chamber procedure, subjects will be lying back on a bed in a small room with plexiglass windows. The subjects will then be asked to relax and breathe normally during the entirety of the testing. During the testing, the study staff will be able to see and hear participants so should they, at any time during the testing, become uncomfortable in the chamber, they may choose to stop the procedure.

Eating Behavior Questionnaires
There is a small potential psychological risk associated with some questions that refer to frequency of induced vomiting, distress associated with overeating. The questionnaires included in this study are frequently used and validated questionnaires in nutrition research, including research in eating disorders and disordered eating. They are important in assessing several unique aspects of eating behavior that could influence our study outcomes.

Loss of Confidentiality

There is a small risk of loss of confidentiality of data during the study. Please see sections 9.0, 17.2, and 19.0 for more details about these risks and also steps taken to mitigate risk.

17.2 Indicate the measures you will use to minimize risks and monitor subjects for safety. (e.g., asking a subject at regular intervals to rate how they are feeling from 1 to 10, or to slowly crouch in order to check their balance.)

Indirect calorimetry: All indirect calorimetry measurements will be performed by trained personnel. Subjects who become uncomfortable over the course of the measurement can withdraw at any time.

MRI: In order to minimize noise from the MRI scanner that can be potentially harmful for the subject, all subjects will be required to wear ear protection while in the scanner. The subject may communicate with the experimenter at any time: 1) before, in between, or after a scan via an intercom device between the scanner and control room, and 2) during a scan via a pneumatic squeeze bulb located in the scanner that triggers an alarm in the control room. Subjects who report discomfort and wish to discontinue their participation will be immediately withdrawn from the scanner. Subjects who are withdrawn from the study will be compensated for their participation to that point.

Advanced MR/Operator Training: Required for those persons wishing to conduct research on the HNL MRIs. Training will consist of presentations, observation, and hands-on practice. Topics will include safety and emergency procedures, subject screening and preparation, scanner set-up and operation, and troubleshooting. Each person must also complete at least 4 hours of observation (to include 2 different studies) and at least 16 hours of supervised scanning (to include 4 different studies). Certification will be granted upon completion of the training and passing of a competency exam. Certified operators will conduct each scanning session.

IV Catheter Blood Draws: To reduce the risks associated with inserting an IV catheter and drawing blood from the catheter, only skilled medical technologists, nurses and/or other medical professionals will insert the IV catheters and draw the blood samples. In addition, a maximum of three attempts will be made to insert the catheter in the participants' arm during a given session.

Fingerstick Blood Sample
The risks associated with performing a fingerstick to collect a capillary blood sample are minimal. The likely risks involve pain and bleeding in the fingertip at the collection site. Rarely, an infection may occur at the puncture site. To minimize these risks, research team members will be trained on the proper technique for obtaining a capillary blood sample and care for the site after the sample is obtained.

Eating Behavior Questionnaires

There is a small potential psychological risk associated with some questions that refer to frequency of induced vomiting, distress associated with overeating. The questionnaires included in this study are frequently used and validated questionnaires in nutrition research, including research on eating disorders and disordered eating. They are important in assessing several unique aspects of eating behavior that could influence our study outcomes. To mitigate risk, participants will be not be required to answer these questions.

Loss of Confidentiality

There is a small risk of loss of confidentiality of data during the study. However, study personnel will make every effort to minimize this risk. Please see sections 9.0 and 19.0 for more detailed information about steps taken to mitigate risk. Briefly:

All study data will be collected and analyzed by trained research personnel.
Each participant will be assigned a coded designation and all study data to be used in the analysis will be de-identified using this coded designation. The de-identified data will not contain any biographical data that can be linked to study participants.
The "key" to the study code will be stored in a separate database which cannot be accessed without specific authorization.
Other screening documents will be stored on site in a locked cabinet that will only be accessible to authorized personnel.
Only personnel who are direct members of the research team will have access to personally identifiable information.
Data acquired during experiments will be collected and stored on a secure server managed by FBRI.

17.3 If applicable, indicate which procedures might have risks to the subjects that are currently unforeseeable. This will be rare, and usually applicable when testing a new drug or device or a new use of an existing drug or device:

There are no new devices or drugs used in this study.

17.4 If applicable, indicate which procedures might have risks to an embryo or fetus should the subject be or become pregnant:

None of the procedures used in this study have been known to cause risk to a fetus or embryo.

17.5 If applicable, describe risks to others who are not subjects (e.g., collection of sensitive health data that might affect sexual partners if disclosed, mandatory reporting of abuse, DNA testing that might affect family members or relationships):

N/A

18.0 Potential Benefits to Subjects

18.1 Describe the potential benefits that individual subjects might experience from participating in the research. Include the probability, magnitude, and duration of the potential benefits, as this will be useful to the IRB's risk:benefit analysis. Do not include benefits to society or others. Do not list monetary or non-monetary compensation for participation, as this is not a benefit These should be included in section 2 or 3 of this document:

There is no direct benefit from subject participation in this study.

18.2 If applicable, specify that there are no anticipated direct benefits for participants:

There is no anticipated direct benefit to individual participants for participation.

19.0 Data Management and Confidentiality

19.1 Describe procedures that you will use for quality control to ensure validity of collected data:

All data will be collected and analyzed by trained research personnel. They will be trained via MRI safety training, Human Subjects Populations, biohazard safety, and vigorous training in standard laboratory procedures.

19.2 Describe any existing data or biospecimens you will obtain as part of this study. Include:

- *Variables or samples to be obtained*
- *Source of the data or specimens*
- *Your authorization to access or receive the data or biospecimens*

- *Whether the data or biospecimens are publicly available*
- *Whether the data or specimens you receive will contain identifiers*

This study collects new data.

19.3 Describe the steps that you will take to handle and secure study data during data collection, storage, use, and transmission. Include information about training of study staff, authorization of access, password protection, encryption, physical controls, certificates of confidentiality, separation of identifiers and data, etc.:

Each subject will be assigned a coded designation. All study data to be used in analysis will be de-identified using this coded designation, which will contain no biographical data that can be linked to individuals. Personal identifiable information will be stored only in Ripple, a HIPAA-compliant database system designed for the purpose of storing and managing identifiable participant information (see section 9.4 above for full Ripple description). Only personnel who are direct members of the research team will have access to personally identifiable information stored in Ripple. The link between the coded designation and personal information will only exist on this secure platform.

Coded designations will be used to collect study data on the FBRI instance of REDCap, a secure HIPAA-compliant database for data collection. In order to collect data via email survey invitation (i.e., health behaviors questionnaires and take-home beverage consumption compliance surveys), participant email addresses must be stored in REDCap in a specific Survey Distribution Participant List. Once the participant has completed participation or withdrawn from the study, their email address will be deleted from REDCap, thus completely eliminating any link of identifiable information with study data. Email addresses included on this list are not able to be exported or downloaded from REDCap as data; they are only held within the Survey Distribution Tools function of REDCap.

MRI images will be stored on secured FBRI and VT ARC servers, and metabolic chamber measurement data will be stored on secured FBRI servers. Behavioral and clinical data will be collected and recorded using FBRI's instance of REDCap via computer or tablet, which is backed up on an FBRI IT-managed secure server.

All staff are informed of and trained on HIPAA Security and Privacy rules.

19.4 For multi-site studies, describe how data or specimens will be handled and secured for each site (e.g., central or disseminated data storage, data coordinating center):

This is a single site study

19.5 Describe the plan for data disposition following the conclusion of the study (e.g., long term maintenance of data, data destruction methods).

- *What information will be included in the long term storage of data or specimens?*
- *How long will the data or specimens be stored?*
- *Where and how data or specimens will be stored?*
- *Who will have access to the data or specimens during long term storage?*
- *Who is responsible for receipt or transmission of the data or specimens?*
- *How will data or specimens be shared or transported?*
- *When and how will personal identifiers be destroyed?*

Data and specimens may be archived indefinitely for future analysis. All data and specimens will be de-identified, coded and contain no specific biographical information which can be related back to an individual (see sections 9.2, 9.4, and 19.3 for more details). The 'key' will be stored in a database (i.e., Ripple), which cannot be accessed unless specifically authorized. Deidentified data may be made available publicly upon publication or study completion, as required by funding agencies and journals. All staff are informed of HIPAA Security and Privacy rules.

20.0 Provisions to Protect the Privacy Interests of Subjects

20.1 Describe the steps that you will take to protect subjects' privacy interests. "Privacy interest" refers to a person's desire to place limits on with whom they interact or to whom they provide personal information (e.g., collecting the minimal amount of private information required to complete the study, protecting the data once it is obtained):

Only the information required to complete the study, document participation or compensate the participant will be collected. Once obtained, these data will be stored securely, accessible only by authorized personnel. For instance, documents with a participant name (i.e., signed Informed Consent Document, MRI Safety Screening form) will be stored in a separate, locked file cabinet from any files containing participant coded identifiers. Keys will only be accessible to authorized staff requiring access, such as trained research coordinators interacting with the participant for the study session.

20.2 Describe steps that you will take to make subjects feel at ease with the research situation in terms of the questions being asked and the procedures being performed. "At ease" does not refer to physical discomfort, but the sense of intrusiveness a subject might experience in response to questions, examinations, and procedures (e.g., use of a same gender investigator to place sensors on the torso, a private changing area if clothing must be changed, sensitivity when discussing pregnancy testing with subjects, making it clear on surveys that participants can discontinue at any time, not asking questions about private or sensitive issues unless necessary for the research):

Research staff will be trained to check with participants throughout all procedures to ensure they are comfortable with all proceedings. Before all procedures, participants will be reminded they can stop at any time. After obtaining written consent for the study, research staff will always ask for verbal consent before each procedure.

20.3 Describe how you plan to access existing sources of information about the subjects (e.g., medical records, grades) and how you will protect participant privacy through the data security plan:

We will not access existing sources of information.

20.4 Describe any required reporting that might occur as a result of your research questions, study populations, and data collection methods. Examples for Virginia and Virginia Tech include:

- ***Any*** *suspicions (e.g., circumstantial, disclosed) of child abuse (physical, emotional, sexual) and neglect*
- *Sexual discrimination and/or sexual violence that involves a student*
- *Disclosure or signs of intention to harm oneself (i.e., suicidal ideation and/or plan)*
- *Disclosure or signs of desire to harm others (i.e., homicidal ideation and/or plan)*
- *Suspected abuse, neglect or exploitation of vulnerable adults (e.g., individuals with a disability, elderly persons)*

We do not foresee any circumstance where this type of required reporting would come up during our study.

21.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

Safety monitoring is <u>required</u> when research involves greater than minimal risk and is sometimes appropriate for other studies.

21.1 Describe:

- *The plan to periodically evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe (e.g., periodic reporting to the IRB, establishing a data monitoring committee, reporting data monitoring committee findings to the IRB and the sponsor).*
- *What data you will review, including safety data, unexpected events, and data that show the ability to produce the intended results.*
- *How the safety information will be collected (e.g., with case report forms, at study visits, by telephone calls with subjects).*
- *The frequency of data collection, including when safety data collection starts.*
- *Who will review the safety data and with what frequency.*
- *The statistical tests for analyzing the safety data to determine whether harm is occurring.*
- *Any conditions that will trigger an immediate suspension of the research (e.g., a serious adverse event).*

The Principal Investigator (PI) or study staff will be responsible for ensuring participants' safety on a daily basis and for reporting Serious Adverse Events and Unanticipated Problems to the Institutional Review Board. The PI will be informed of serious adverse events as soon as they occur by the study staff and will report any serious adverse events and unanticipated problems to the IRB. Safety information will be collected at study visits by research personnel. This safety information will be reviewed weekly by the PI during a weekly lab meeting. A serious adverse event would trigger immediate suspension of the research.

22.0 Compensation for Research Related Injury

22.1 If the research involves more than minimal risk to subjects, describe the available compensation in the event of research-related injury, if any:

In the event that a research-related event or injury does occur, participants will be treated appropriately according to the nature of the event or injury. Generally, care will be billed to the participant, their insurance, or another third party. If participants are no longer able

to continue in the study, they will receive compensation in accordance with the type and number of sessions completed.

22.2 Provide a copy of contract language, if any, relevant to compensation for research-related injury. At Virginia Tech, this is most common for sponsored research:

This study does not present more than minimal risk to the subjects.

23.0 Economic Burden to Subjects

23.1 Describe any costs that subjects might be responsible for because of participation in the research, including any uncompensated costs for items such as transportation, missed work, and childcare:

Participants are compensated for their time. They can plan to participate outside of work and child-care needed studies.

24.0 Consent Process

24.1 Indicate the process by which you will obtain consent for study participation. Please upload all consent, parental permission, and assent forms, documents, and scripts referenced in this section to Protocol Management.

Describe the following:

- *Where the consent process will take place (e.g., clinic waiting area, classroom, online)*
- *The time interval between sharing the consent information with the prospective subject and obtaining consent. For lab, interview, and focus group studies, the Virginia Tech IRB prefers that subjects have at least 24 hours to review the consent form and study information before the appointment where consent will be obtained. For simple online survey studies, you can typically present the consent information immediately before subjects begin participation.*
- *If applicable, processes to ensure ongoing consent or assent (e.g., for multiple sessions; for research in which a minor will turn 18 during the study; for longitudinal research with minors who will later be asked to provide or affirm their assent).*
- *Please review "SOP: Informed Consent Process for Research (HRP-090)" for recommended procedure. Describe your process, being sure to include:*

- *The name and role of all study personnel who will be trained and certified by the PI to conduct the consent process*
- *The time that will be devoted to the consent discussion*
- *Steps that you will take to minimize the possibility of coercion or undue influence*
- *Steps that you will take to gauge or ensure the subjects' understanding*

Consent for the screening questionnaire will be documented by completion of the survey by the participant. Information about the study, instructions for completing the survey, next steps for participation, and what will happen with their information if they are/are not (i.e. screen fail) determined to be eligible to participate will be provided in the "Introduction" section of the survey (see supporting documents).

During the pilot study (involves a single session) or full study consent session, consent for participating in the study will be obtained on-site by research personnel in a quiet waiting area or private room if the waiting area is in use prior to the study procedures. Consent materials will be emailed to the participants for their review at least 24hrs in advance of their first appointment.

Participants will be given as much time as they request to review the consent documents.

This study involves multiple sessions and a single consent form will be signed that details all sessions. Participants will provide their consent for all sessions at the first session, but will be informed they may withdraw from the study at any time. Verbal consent will be obtained before each subsequent session.

Staff will devote as much time as requested to the consent process. Typically, review of the consent form takes approximately 10 minutes. Participants will then be given time to review the form independently and ask any questions they may have. Staff will confirm with the participant that they have had all questions answered prior to the participant signing the consent form.

In order to determine understanding, staff will discuss each section with the participant, verifying their understanding by verbal confirmation throughout.

Non-English Speaking Subjects

- *Indicate what language(s) other than English are understood by prospective subjects or representatives.*
- *If non-English speakers will be recruited, describe the process you will use to ensure that the oral and/or written consent information provided will be in a language that they understand.*

- *If you translate consent forms and study materials, please provide a certified translation of the form as well as the certification document.*
- *Indicate the spoken language that study personnel obtaining consent will use. Describe how you will assess fluency of personnel obtaining consent to ensure that the translation is accurate.*

We will recruit only English-speaking participants.

Waiver or Alteration of Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception)

- *Review the "CHECKLIST: Waiver or Alteration of Consent Process (HRP-410)" to ensure you have provided sufficient information for the IRB to make these determinations (i.e., that it meets the criteria for a waiver or alteration of the consent process).*

We do not waive consent.

Subjects who are not yet adults (minors: infants, children, teenagers)

- *Describe the criteria that you will use to determine legal age for consent to treatments or procedures involved in the research under the applicable law of the jurisdiction in which the research will be conducted (e.g., in Virginia, individuals under the age of 18 years).*
  - *For research conducted in Virginia, review "SOP: Legally Authorized Representatives, Minors, and Guardians (HRP-013)" to determine which individuals in the state meet the definition of "minor."*
  - *For research conducted outside of the state, please describe the legal requirements for the definition of "minor."*
- *Describe the process for obtaining parental permission.*
  - *Permission from one parent is acceptable for studies that involve no greater than minimal risk OR involve greater than minimal risk but present the prospect of direct benefit to the minor subject.*
  - *Permission from both parents is required in all other cases (unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the minor).*

- *Describe whether you will obtain permission from individuals other than parents or Legally Authorized Representatives, and if so, who will be allowed to provide permission. Describe the process you will use to determine these individuals' authority to consent to the minor's general medical care.*
- *Indicate whether you will obtain assent from all, some, or none of the minors. If you will obtain assent from some minors, indicate which minors will be required to assent. Consider chronological age and intellectual capacity when determining who will be required to provide assent (e.g., infants are unable to assent. However, teenagers are likely able to read and sign an assent form).*
- *When assent of minors is obtained, describe whether and how you will document it. Will minors sign an assent form or give verbal assent?*
- *Attach parental permission and minor assent forms or scripts in Protocol Management.*

Only adults 18 years or older will be eligible to participate in the study.

Adults Unable to Consent

- *Describe the process you will use to determine whether an individual adult is capable of consent.*
- *List the individuals from whom you will obtain permission in order of priority (e.g., durable power of attorney for health care, court appointed guardian for health care decisions, spouse, and non-minor child).*
  - *For research conducted in the Virginia, review "SOP: Legally Authorized Representatives, Minors, and Guardians (HRP-013)" to determine which individuals in the state meet the definition of "legally authorized representative."*
  - *For research conducted outside of Virginia, please describe the legal requirements for obtaining permission from a legally authorized representative in the state where the research will occur.*
- *Describe the process for assent of the subjects.*
  - *Indicate whether you will require assent from all, some, or none of the subjects. If some, indicate which subjects will be required to assent and which will not.*
  - *If you will not obtain assent from some or all subjects, please provide justification for not obtaining assent.*
  - *Describe whether and how you will document assent.*

Adults must be able to consent themselves.

25.0 Process to Document Consent in Writing

25.1 Consult "SOP: Written Documentation of Consent (HRP-091)" for recommended procedures, and describe whether and how consent of the subject will be documented in writing:

Written consent will be obtained from the participants during the consent session described above. Participants will be emailed the informed consent document ahead of time so they may review it. During the consent session, two copies of the consent document will be provided to the participant to sign: one they will be given to keep for their own records and one copy will be kept by research personnel in a locked filing cabinet.

25.2 If the research presents no more than minimal risk of harm to subjects and involves no procedures for which written documentation of consent is normally required outside of the research context, you can request that the IRB waive the requirement to obtain written documentation of consent (e.g., consent to participate is indicated by pressing a button for an online questionnaire – after the consent information is presented and before the questionnaire begins):

We do not request to waive written consent.

25.3 If you will document consent in writing, attach a consent document with places for signatures. If you will obtain consent, but not document consent in writing, please attach the consent script or text. Review "CHECKLIST: Waiver of Written Documentation of Consent (HRP-411)" to ensure that you have provided sufficient information. You should use "TEMPLATE CONSENT DOCUMENT (HRP-502)"to create the consent document or script:

Consents for this project are included in supporting documents.

26.0 Resources Available

26.1 Describe the resources available to conduct the research. For example, as appropriate:

- *Describe the PI's availability to supervise the research.*

- *Justify the feasibility of recruiting the required number of suitable subjects within the agreed recruitment period. For example, how many potential subjects do you have access to? What percentage of those potential subjects do you need to recruit?*
- *Describe the time that you will devote to conducting and completing the research.*
- *Describe your facilities.*
- *Describe the availability of medical or psychological resources that subjects might need as a result of an anticipated or unanticipated consequence of participation in the research.*
- *Describe your process to ensure that all persons assisting with the research are adequately informed about the protocol, the research procedures, and their duties and functions (e.g., training plans, detailed study notebooks).*

The PI is a research-dedicated (100%) Faculty member at Virginia Tech and the Fralin Biomedical Research Institute. The majority of the PI's time is spent supervising and analyzing ongoing research.

Fralin Biomedical Research Institute

Dr. DiFeliceantonio's lab is part of the Fralin Biomedical Research Institute at Virginia Tech Carilion (FBRI at VTC), located in Roanoke, Virginia. The Research Institute was founded approximately eight years ago as part of a commitment to biomedical sciences by Virginia Tech, and was originally called the Virginia Tech Carilion Research Institute. The name changed to Fralin Biomedical Research Institute at VTC in late 2018 following a transformative gift of fifty million dollars by the Fralin Family. Primary institute faculty have a primary faculty appointment within a traditional department at Virginia Tech, but are funded through and housed at the FBRI. Primary faculty comprise a broad interdisciplinary group working in a variety of areas such as neural computation, psychiatric and developmental disorders, genetics, substance abuse and molecular virology and biology. The FBRI also provides to the faculty an administrative staff of individuals with expertise in grants management, personnel management, supply ordering/tracking, web systems management, desktop support, laboratory animal care, IRB support, software development and facilities and operations support and planning as well as general administrative support.

The Human Neuroimaging Laboratory serves as the primary human imaging facility of the Fralin Biomedical Research Institute (FBRI). The Human Neuroimaging Laboratory has two 3T Siemens MRI machines (one a Trio and one a Trio recently upgraded to Prisma) within the primary facility, as well as another Trio at an outpost installation near the main campus in Blacksburg, VA to take advantage of the large subject population.

The Fralin Biomedical Research Institute is sited in an approximately 100,000 square feet building, divided among:

32 faculty offices
~ 30,000 sqft of dry-lab space
~ 46,000 sqft of wet-lab space
~ 23,000 sqft of administrative/office space
30 behavioral testing rooms
2 observation rooms with video/audio recording
4 conference rooms with AV / video conferencing capabilities
1 data center with a 16 rack capacity
3 MRI scanning suites (includes 1 offsite location)
In 2020 a new ~140,000 sqft building will open adjacent to the existing building and will house additional faculty, staff, and laboratories.

Computing Resources:

The Fralin Biomedical Research Institute (FBRI) at Virginia Tech Carilion (VTC) houses the following shared computing resources available:
Dell / Intel HPC Cluster (20 nodes, 960 Core, 3,840 GB RAM, 40Gb Interconnect) – SLURM; IBM iDataPlexLinux Cluster (60 node, 720 core Intel Xeon based, 1,440GB RAM, GigE Interconnect) - PBS; 10+ dedicated Linux servers (48 core AMD Opteron-based, 192GB RAM) are available for image and data analysis; Virtualized infrastructure using Vmware vSphere, virtual machine environment available for general compute and image and data analysis; 40Gbit/s storage connectivity for research data; 10 Gbit/s Internal Local Area Network between file servers and cluster; 8Gbit/s Fibre Channel Storage Area Network; 10 Gbit/s Wide Area Connection for access to Virginia Tech main campus / Internet; 1.4 Petabytes of NAS centralized disk storage; 250 Terabytes of SAN-attached centralized disk storage; 400 Terabyte library-based tape backup; Nightly backups and snapshots; 2.4Ghz / 5Ghz secure wireless network; 1 Gbit/s commodity Ethernet network; Data Analysis Tools: MATLAB, SPM12, AFNI, FSL, MRIcro, xjView, R, SAS, Prism Graphpad, SPSS; Productivity Tools: Adobe Suite, Microsoft Office Suite, vi, vim, emacs

MRI Scanning Resources (FBRI):
Virginia Tech has three research-dedicated Siemens 3T MR scanners (2 Siemens Magnetom TIM Trios, and 1 PRISMA-FIT) available. Each scanner bay is equipped with the following stimulation and response interfaces:
behavioral response: two-hand, eight-button optical response pads with USB, serial, and TTL output (Current Designs, Inc.)
video stimulation: rear-projection video display (Hitachi CP-SX635)
corrective lenses for use with video stimulation: MR-compatible frames with insertable polycarbonate lenses (prescriptions range from -8.00 to +8.00) (Solo Bambini)
stimulus delivery: dedicated computers for experiment presentation (Dell Optiplex 980)
audio delivery: MRI compatible headphones or intercom system.

Metabolic Chamber Resources (FBRI):

Virginia Tech has 2 research-dedicated chambers of whole-room indirect calorimetry available (MEI Research, Ltd). The larger room is equipped for 24-hour measurements, and the smaller room is equipped for shorter-term measurements.

27.0 Multi-Site Research

Contact the HRPP for multi-site research (involving multiple institutions) and the details required for this section will be provided. Otherwise, indicate N/A.

This is a single site study.