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 role of altered nutrient partitioning in food reward

PROTOCOL NUMBER:

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

#21-964

PRINCIPAL INVESTIGATOR:

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Is Virginia Tech the primary awardee or the coordinating center of this grant or contract? If not, list the primary institution: University of Virginia

VERSION NUMBER/DATE:

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

2.0

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	10/12/22	Section 6.0: updated information about where and how recruitment materials will be used Section 11.0: Updated details about blood draw protocol to be consistent with the types of blood tubes we are able to procure Section 11.0: updated compensation amounts for each visit to equal ~$20/hr	
2	4/26/23	Section 12.0: Inclusion Criteria: changed inclusion criterion for fMRI scan from BMI 25-35 kg/m2 to BMI >25 m/kg2	no

Table of Contents

1.0 Study Summary

Study Title	The role of altered nutrient partitioning in food reward
Study Design	This is a within-subjects crossover study design that involves a conditioning paradigm in which participants are exposed to novel flavored beverages paired with sugars and non-nutritional sweeteners, and assessment of energy expenditure and blood metabolites will be performed during the conditioning paradigm. A subset of participants will undergo fMRI measurements and metabolic chamber measurements.
Primary Objective	To assess the feasibility of measuring reward response following a flavor-nutrient conditioning paradigm across the normal to obese body mass index range and in states of altered metabolic health
Secondary Objective(s)	To develop an appropriate protocol for measuring metabolic flexibility in a "flex" metabolic chamber
Study Population	The general US population
Sample Size	15
Research Intervention(s)/ Investigational Agent(s)	Blood measurements, resting and postprandial energy expenditure and substrate oxidation, questionnaires, magnetic resonance imaging
Study Duration for Individual Participants	6-7 weeks in total: Week 1: Training Session (1 hour) Weeks 2-4: Behavior Sessions (4 sessions x 2.5 hours each) Week 5: Post-testing Session with fMRI (only a subset of participants; 3 hours) Week 5: Post-testing Session without fMRI (a majority of participants; 1 hour) Weeks 6-7: Metabolic chamber sessions (2 sessions x 6 hours each, with 5-day washout between sessions) 4 days total of controlled feeding with food provided by the metabolic kitchen
Acronyms and Definitions	REE: resting energy expenditure; MRI: magnetic resonance imaging; FBRI: Fralin Biomedical Research Institute; CHBR: Center for Health Behavior Research

2.0 Objectives

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

The first aim of this study is to assess the feasibility of measuring reward response following a flavor-nutrient conditioning paradigm across the normal to obese body mass index range and in states of altered metabolic health. The second aim is to develop a protocol for measuring metabolic flexibility in a "flex" metabolic chamber over a 6-hour measurement.

2.2 State the hypotheses to be tested:

We hypothesize that reinforcement learning will be impaired with increasing obesity and that metabolic flexibility can be determined utilizing data produced from a 6-hour measurement inside a "flex" metabolic chamber.

3.0 Background

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

There is conflicting evidence as to whether people with obesity find foods more reinforcing. People with obesity exhibit a greater motivation to consume palatable foods that does not habituate over time compared to healthy weight individuals (Saelens and Epstein 1996; Epstein et al. 2007; Temple et al. 2009); however, they do not report liking food more (Wall et al. 2020). Obesity is also associated with decreased sensitivity to post-ingestive signals (Beutler et al. 2020), possibly due to dietary history and exposure (Small and DiFeliceantonio 2019). Further, there is evidence for impaired reinforcement learning and habituation in studies from both humans with obesity and animals (Kroemer and Small 2016; Coppin et al. 2014; Reichelt, Morris, and Westbrook 2014; Johnson and Kenny 2010). Additionally, many hormones that are altered in states of obesity and metabolic dysfunction, such as insulin, influence food reinforcement. For example, a recent study in humans revealed central insulin signaling influences striatal dopamine tone (Kullmann et al. 2021). However, there is no experimental evidence in humans that post-ingestive learning is altered in states of obesity. The experiments in this proposal aim to fill that gap.

The normal process of energy metabolism involves a dynamic and coordinated switch between primarily fat and carbohydrate as oxidative fuel substrates, depending upon nutritional or physiological condition. This process of adjusting substrate oxidation to match substrate availability and energy demand is termed “metabolic flexibility” (Kelley and Mandarino 1990). Metabolic inflexibility, on the other hand, is caused by nutrient overload in the mitochondria and results in altered nutrient partitioning in which a consistent mix of oxidative substrates, regardless of nutritional or physiological

circumstances, is utilized (Muoio 2014). Metabolic inflexibility has been identified in obesity (GM et al. 2012; Hulver et al. 2003), type 2 diabetes(Kelley and Mandarino 1990), insulin resistance (Kelley et al. 1999; van der Kolk et al. 2016), and other conditions characterized by dysregulated energy homeostasis. Metabolic inflexibility is putatively a key influence in the pathogenesis of peripheral insulin resistance, as biomarkers of altered fat oxidation have been observed prior to the development of insulin resistance (Jans et al. 2011; Heilbronn et al. 2007). If flavor nutrient conditioning is dependent upon some mechanism involved in the pathway of nutrient sensing, transport, and utilization, altered nutrient partitioning due to metabolic inflexibility may provide insight into the relationship between post-ingestive metabolic signals and food reward in obesity and metabolic disease.

Indirect calorimetry is a powerful tool for determining energy expenditure and macronutrient oxidation in response to a mixed-macronutrient diet or meal. In other words, it allows for a versatility in measurement of metabolic flexibility to both fat and carbohydrate in a physiologically relevant manner. Indeed, a new method for assessing metabolic flexibility to a high-carbohydrate or high-fat meal using indirect calorimetry in a large, whole-room metabolic chamber has recently been published (McDougal et al. 2020); however, the reproducibility of this method across metabolic chambers and clinical populations has not yet been determined. Furthermore, this methodology is designed around use of a 27 m3 chamber, which requires a relatively long duration of measurement for an appropriate temporal resolution of measured O2 and CO2 concentrations to determine energy expenditure and macronutrient oxidation (Chen et al. 2018). The Fralin Biomedical Research Institute houses two state-of-the-art metabolic chambers. The smaller of the two chambers, approximately 4.6 cubic meters, allows for more rapid and accurate measurement of O2 and CO2 compared to large chambers and is specifically designed for conducting dynamic metabolic studies over shorter measurement durations. This smaller “flex” chamber is one of only a few in the world, and as such, no methodologies to assess metabolic flexibility specifically in the “flex” chamber have been developed. Thus, we are poised to take advantage of novel technology to lead the development of a new method to assess a key component of metabolic health.

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:

Although substantial advances in obesity treatment have been made in recent decades, obesity remains a public health epidemic. This may in part be due to the fact that although current treatments are effective in improving metabolic health and reducing body weight in the short term, strategies that promote long-term maintenance of metabolic health and reduced body-weight remain elusive. Obesity is therefore a chronic disease that requires lifelong attention. From a public health perspective, developing long-term effective treatments is an essential medical research goal. The gut-brain axis has long been recognized as an important regulator of metabolism and energy homeostasis; however, only recently have advances in research tools allowed for a better understanding of the complex mechanistic integration of gut and brain signals. The fidelity of the gut-brain axis presents a novel and potentially powerful therapeutic target for obesity and metabolic diseases. In this proof-of concept pilot, we propose to probe the gut-brain axis as a target for obesity intervention by testing the fidelity of the gut-brain axis 1) across a range of body mass index (BMI) and 2) in states of altered metabolic health (independent of BMI). The data from this proposal will provide evidence for the gut-brain axis as a therapeutic target in obesity treatment and allow the development of state-of-the-art tools to measure metabolic health at Virginia Tech, essential steps towards applying for federal funding.

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 blood metabolite measurements, indirect calorimetry measurements, drink preference and ratings, 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):

This study consists of two main aims. In the first aim, a within-subjects design will be used to compare the rewarding properties of two beverages, one containing 75kcal of sugar and one containing 0 kcal sucralose in individuals with normal weight and with excess adiposity or high body mass index. In a subset of participants, neural activation to each of the beverage tastes measured by fMRI will be compared. In the second aim, a within-subjects design will be used to compare metabolic response to a high-carbohydrate and high-fat test meal using a 6-hour metabolic chamber stay.

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):

The first aim will use linear mixed effects models to compare ratings of the beverages and control for confounding variables. Linear regression will be used to relate blood and energy expenditure measures to liking and other subjective ratings. Standard preprocessing and statistical techniques for fMRI data will be used to compare beverages and look for relationships between neural activity and physiological measures. The second aim will use linear mixed effects models to compare energy expenditure and substrate oxidation to the test high-carbohydrate and high-fat meals measured in the metabolic chamber. Measures of metabolic flexibility will include changes in respiratory exchange ratio in response to each meal, the time to reach peak respiratory exchange ratio after meal consumption, slope from baseline to peak respiratory exchange ratio, and rate and slope of the return to baseline respiratory exchange ratio.

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.*

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. 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.

The study procedures for the study will all take place at the Fralin Biomedical Research Institute (FBRI) at VTC campus in Roanoke, VA. The consent session and metabolic cart measurement sessions will occur in the Center for Health Behavior Research (CHBR) at the FBRI. Flex calorimeter sessions and controlled feeding will occur in the metabolic chamber and metabolic kitchen, respectively, at the FBRI at VTC.

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:
This study will involve the use of 2 beverage solutions, which will be given to each participant. Beverages include citric acid, water, flavor, and either:
1. 0 kcal sucralose (to match the sweetness of the calorie-containing sugar beverage)
2. 75kcals sugars

Sucralose, sugars, and citric acid are common food and beverage additives that nearly every participant would have consumed previously in their normal diet.

The flavors used will be 0.002% acerola, 0.5% bilberry, 0.1% horchata, 0.1% lulo, 0.2% yuzu. 0.1% papaya, 0.1% chamomile, 0.1% aloe vera, 0.1% mamey, and 0.2% maqui berry (Bell Labs Flavors and Fragrances,IL, USA, product numbers: 33.81940, 15.80182, 132.81478, 141.14606. 101.29478, 102.82506, 141.31243, 141.31480, 46.29969 and 13.32059).

Foods:
This study will involve consumption of high carbohydrate (60% carbohydrate, 20% fat) and high fat (60% fat, 20% carbohydrate) test meals. In addition, a standardized diet (50% carbohydrate, 30% fat) will be provided for 2 days preceding each test meal measurement day. All of the foods included are typical foods nearly every participant would have consumed in their normal diet. Participants will be screened for food allergies/intolerances and excluded if any of these foods are included in the study diet or test meals.

Device Handling:
This research involves use of a metabolic chamber and metabolic cart. Both are located in spaces with restricted, proxy access. This research also involves the use of an MRI scanner. The MRI scanner is located in a space in the Human Neuroimaging Lab with 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 this 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:

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

Magnetic Resonance Imaging: A subset of 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.

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 crossover design and within-subjects analysis plan where participants receive all conditions during the flavor-nutrient conditioning paradigm of Aim 1. A subset of participants will also undergo fMRI scanning for Aim 1. All participants will undergo the controlled feeding diet and metabolic chamber measurements for Aim 2.

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.*

Study Overview:
Approximately 15 people from the general population will be recruited for Aim 1 (without fMRI) and Aim 2 of this randomized crossover design study conducted at FBRI. Of these 15 people, 5 will also undergo fMRI scanning as a part of Aim 1. This will be an approximately 6- to 7-week study. Participants will report to the research laboratory in Week 1 for the consent and training session. They will return to the research laboratory to complete 2 behavior sessions per beverage and a final post-testing session in Aim 1. Participants who are asked to complete the fMRI scan will undergo this measurement during the final post-testing session. For Aim 2, participants will report to the metabolic kitchen at FBRI for 2 days preceding each chamber measurement day to collect food to be consumed for each of those days. On each metabolic chamber measurement day, they will report to the metabolic chambers located in 4 Riverside. There will be a 5-day washout period between the first metabolic chamber session and controlled feeding for the second metabolic chamber session.

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. 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.

AIM 1:

Consent and Training Session
Informed Consent
Participants will be asked to sign a consent form upon arrival to the CHBR. They will be given ample time to review and ask questions prior to signing the document. After research personnel have answered the participant's questions and the consent form has been signed, participants will begin the training session.

Anthropometrics and Clinical Measures
Participants' height and weight and hip and waist circumferences will be measured. A fingerstick blood sample will be collected for point-of-care hemoglobin A1C assessment to ensure eligibility. Blood pressure will be collecting using an automated sphygmomanometer. Body composition will be assessed using bioelectrical impedance analysis.

Flavor Ratings
The participants will first be trained by the researcher on how to use the standard perceptual rating scales (Bartoshuk et al., 2004). 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 two flavors that were equally liked and equally unfamiliar to the participant. These flavors will be used in the beverages the participants will consume during the behavioral conditioning sessions (one beverage per 2 sessions). In addition, participants will complete a triangle test to determine whether they can detect a difference between sugar- and sucralose-sweetened beverages.

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 (i.e., Aim 1 without the fMRI scan and Aim 2).

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 prior to the first behavior session. It is estimated that it will take the participant approximately 30 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. The automated invitations will also contain information about how to contact appropriate lab personnel to opt out of further participation if a participant wishes to do so.

Behavior Sessions 1 and 3 (interchangeable with Behavior Sessions 2 and 4, respectively):
Participants will come to the CHBR after a 4 hour fast. Participants will also be asked to abstain from moderate-to-vigorous physical activity for at least 24h prior to their session. They will first rate their internal states on standard scales.

An IV catheter will then be placed in participants' arms for blood draws at seven time points during the 1-hour session: at baseline and 10-, 15-, 20-, 30-, 40-, and 60-minutes of the session. For the IV catheter placement, a small plastic tube will be placed in one of the veins in the medial cubital fossa area of the arm; this tube will stay in place for the duration of the 1-hour testing session. No more than three attempts will be made to insert the IV catheter during each blood draw testing session. Once the catheter is inserted, a baseline sample of 2 tubes of blood will be drawn: a 4 ml tube for EDTA plasma, and a 5 ml tube for serum. In the case of difficulty inserting the IV catheter and obtaining blood at the baseline time point, we will evaluate whether to continue the protocol or not. If the participant is willing, venous blood draw samples may be collected instead at baseline, 30, and 60 minutes.

Participants will then consume a single 355ml conditioning beverage (with the appropriate conditioning stimuli and flavor as determined during the consent and training session) within 5 minutes and rate internal states immediately after consumption. Two tubes of blood (one 4 ml EDTA plasma, one 5 ml serum) will be collected at 10-, 15-, 20-, 30-, 40-, and 60-minutes following beverage consumption. In total, 14 tubes of blood will be drawn during the session (approximately 63 mls of blood). The IV catheter line will be flushed with normal saline between each blood draw to prevent coagulation in the catheter tubing. Once the 60-minute sample has been drawn, the catheter will be removed and the venipuncture site secured with sterile tape or a bandage.

At the end of this session, participants will be given 2 additional bottles containing 355 ml of the beverage consumed during this session. Participants will be instructed to consume one beverage 1 hour before dinner that evening and the other beverage 1 hour before lunch the following day. Participants will also be asked to complete a short survey regarding consumption of each of these beverages. An automated email containing links to a survey about the dinner and lunch beverage consumption will be sent using the same REDCap automated email function described above.

Behavior Sessions 2 and 4 (interchangeable with Behavior Sessions 1 and 3, respectively):
Participants will come to the CHBR after a 4 hour fast. Participants will also be asked to abstain from moderate-to-vigorous physical activity for at least 24h prior to their session. This session will occur within the same week as Behavioral session 1 (or 3, as appropriate) with at least one day in between the sessions. They will first rate their internal states on standard scales.

Participants will then rest for approximately 30 minutes in preparation for the fasting indirect calorimetry measurement, which requires participants to be in a calm, restful state for accurate measurement. Participants will then undergo an indirect calorimetry measurement using a metabolic cart for approximately 30 minutes.

Indirect calorimetry determines gas exchange (i.e., oxygen consumption and carbon dioxide expiration) at the level of the lungs, and these measures are used to calculate energy expenditure and fat and carbohydrate utilization. During the test, participants lie supine while breathing normally into a transparent plastic hood. There are two holes on the hood, one that allows the inflow of room air into the hood, and another that allows for the extraction of gases into the gas analyzer via a vacuum tube (see picture below). The hood is then sealed around the participant's shoulders to prevent any gas from escaping during the test. Indirect calorimetry using a metabolic cart is a common, noninvasive assessment conducted routinely in both research and clinical nutrition practice. There is no more than minimal risk associated with this method of indirect calorimetry, and participants may wear normal clothes during the procedure. Since the hood is clear plastic, participants will be able to see and communicate with research personnel throughout the test. Should a participant become uncomfortable with the test, they may choose to stop the procedure at any time.

Following completion of the approximately 30-minute fasting indirect calorimetry measurement, participants will be given a 355 ml conditioning beverage (with the appropriate conditioning stimuli and flavor as determined during the consent and training session) to consume within 5 minutes. Participants will then rate their internal states on standard scales, and indirect calorimetry will be performed again for approximately 1 hour.

At the end of this session, participants will be given 2 additional bottles containing 355 ml of the beverage consumed during this session. Participants will be instructed to consume one beverage 1 hour before dinner that evening and the other beverage 1 hour before lunch the following day. Participants will also be asked to complete a short survey regarding consumption of each of these beverages. An automated email containing links

to a survey about the dinner and lunch beverage consumption will be sent using the same REDCap automated email function described above.

Post-Testing Session (for participants NOT undergoing fMRI portion of the protocol):
Participants will come to the CHBR after a 4 hour fast. Participants will also be asked to abstain from moderate-to-vigorous physical activity for at least 24h prior to their session. They will first rate their internal states on standard scales.

They will then taste and rate on standard scales the 2 flavors they consumed as part of the conditioning beverages throughout the protocol. After the rating, they will be given access to bottles containing the two they consumed during the protocol sessions and allowed to drink as much or as little as they want from the 2 bottles for 30 minutes. Consumed amounts of each beverage will be measured and recorded. Lastly, they will be offered 2 additional bottles containing the same conditioning beverages and allowed to choose one to take home with them in a forced-choice test.

Post-Testing Session (for participants who ARE undergoing fMRI portion of the protocol):
Participants completing the fMRI portion of the protocol will first report to the CHBR after a 4 hour fast. Participants will also be asked to abstain from moderate-to-vigorous physical activity for at least 24h prior to their session. They will first rate their internal states on standard scales. Then, they will be escorted to the Human Neuroimaging Laboratory in 2 Riverside for the fMRI scan.

During this scan, participants will lie on a table that slides into the bore of the scanner. Using a head coil, the functional images will be acquired using an EPI bold sequence. Because the process is noisy (due to the sounds generated as the magnetic field gradients are changed), participants will wear a pair of headphones and disposable earplugs. During the entirety of the scan, subjects will be able to communicate with the experimenter via a speaker and microphone system. If subjects become uncomfortable or claustrophobic while in the scanner, they will be able to squeeze an emergency ball to get the attention of the experimenters who will then immediately remove them from the scanner. They can then withdraw from the study if they are unable to complete the MRI.

While in the magnet, subjects will taste small amounts of liquid stimuli that will be presented according to a standard paradigm we have successfully used before in taste MRI studies (Small et al., 2003; Small et al., 2004). 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 subject. The mouthpiece will be anchored to the head coil so that the end comfortably rests on the anterior tongue, just inside the subjects' mouth. This will not interfere with breathing through the mouth and subjects will always be able to breathe through their noses. To ensure participants can breathe through their nose, they will be asked to close their mouth and take one full breath (inhale and exhale) through their nose. If they are unable to breathe normally through their nose on the day of the scan, the scan will be rescheduled. 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). Each taste will be followed by a rinse of a tasteless solution.

After the fMRI is completed, they will be given access to bottles containing the two they consumed during the protocol sessions and allowed to drink as much or as little as they want from the 2 bottles for 30 minutes. Consumed amounts of each beverage will be measured and recorded. Lastly, they will be offered 2 additional bottles containing the same conditioning beverages and allowed to choose one to take home with them in a forced-choice test.

AIM 2:

Controlled Feeding:
Participants will consume a standardized diet (50% carbohydrate, 30% fat) for 2 days prior to each metabolic chamber session. Total daily energy needs for each participant will be estimated using average resting energy expenditure determined during completion of Behavior Sessions 2 and 4 in Aim 1 and an activity factor derived from the physical activity questionnaire completed as part of the Consent and Training Session in Aim 1. The 2-day diet will be developed by a registered dietitian, and all foods will be prepared and assembled in the metabolic kitchen located in 4 Riverside.

Participants will report to the metabolic kitchen to pick up coolers of food. Participants will be provided with all foods and drinks, except water, for the 2 days preceding each metabolic chamber measurement and instructed to consume all foods and beverages provided in the coolers.

Metabolic Chamber Sessions:
Participants will report to the metabolic chamber lab in 4 Riverside after a >8 hour (overnight) fast. Participants will also be asked to abstain from moderate-to-vigorous physical activity for at least 24h prior to their session.

Participants will then enter the flex metabolic chamber, which is outfitted with a bed, pillows, and a TV, to rest quietly during the fasting measurement. The fasting measurement will take approximately 50 minutes. After a fasting measurement, participants will be instructed to consume a high-carbohydrate (60% carbohydrate, 20% fat) or high-fat (60% fat, 20% carbohydrate) test meal within 5 minutes. Participants will then rest inside the chamber, without sleeping, for 5 hours during the post-consumption measurement.

The metabolic chamber operates under the same indirect calorimetry assumptions and principles as the metabolic cart, but the size of the chamber (4.6 cubic meters) allows for longer measurements with better comfort for participants compared with the metabolic cart. There are no known risks associated with this method of indirect calorimetry, and participants may wear normal clothes during the procedure. The chamber is outfitted with a 2-way intercom system, and one wall is clear plastic; participants will be able to see and communicate with research personnel throughout the test. Should a participant become

uncomfortable with the test, they may choose to stop the procedure at any time. (See picture below.)

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:

Indirect calorimetry
There are no known risks associated with either method of indirect calorimetry. However, some people may feel uncomfortable or anxious with the enclosed clear plastic hood placed over their head and shoulders during the metabolic cart measurement. Subjects 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. Some people may feel

uncomfortable enclosed in a sealed room during the metabolic chamber measurement. Subjects will be able to breathe normally during the entirety of the test procedure, and a large window comprises most of one wall of the chamber. Participants will be able to see and communicate with the experimenter during the entirety of the measurement and will be able to tell the experimenter if they would like to terminate the measurement.

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. Subjects who squeeze this bulb 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.

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 in the phlebotomy chair.

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.

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 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 (attached). Perceptual ratings will be collected using a laptop or tablet and custom software written in Matlab. 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. Brain images will be acquired during fMRI scans. Gas exchange values will be collected using a standard metabolic cart from ParvoMedics and a metabolic chamber from MEI Research, Ltd.

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 and 24-hour food recall 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 energy expenditure variables collected during indirect calorimetry measurement will be stored in FBRI's instance of REDCap.Data will be stored indefinitely for future analysis and use in developing future protocols using indirect calorimetry methods. 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. As it is more cost effective to run an ELISA or Immulite 1000 with a full number of wells, these samples will be stored until they can be analyzed in batch. 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:

Only a 4 digit number will be used to label data and the date collected.

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 number. Participant IDs will be created sequentially starting at 9999. The key linking the participant name or identifiable information and the code will be stored on an encrypted server managed by the FBRI IT department. This server can only be accessed by those on the research team. All study data will be kept on a separate server from identifiable information, also encrypted. Any paper documents generated that contain other identifiers, such as name, phone number, or email will be stored in a locked file cabinet accessible only by authorized staff.

Identifiable participant information will be destroyed 5 years after publication or at the written request of the participant.

Per Virginia Tech policy, in order to compensate participants, social security number (or Taxpayer ID) and street address will be collected via a W-9 form or W-8BEN. The participant address and SSN will be entered into the ClinCard systems, 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 any documents with SSN or address used for payment purposes.

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:

Results of the study will not be shared with the participants as they are de-identified prior to analysis and/or publication

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.*

Consent and Training Session: 2 hours
Behavior Sessions 1-4: 2 hours each (8 hours total)
Post-Testing Session without fMRI: 1 hour
Post-Testing Session with fMRI: 3 hours
Metabolic Chamber Sessions 1 and 2: 6 hours each (12 hours total)

Each participant is expected to be enrolled for approximately 8-10 weeks.
The study, including recruitment, enrollment, and primary data analysis, is expected to last 1 year.

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:

Participants will complete an online screening survey after they have indicated interest in participating in the study. A link to the Ripple survey will be sent to participants via email, or they can access the link through a written web address or QR code on our flyer. Additionally, social media posts will contain the web address. Participants undergoing fMRI will also complete an MRI screening form.

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 18.5-40 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. Able to speak and write in English
6. Specific to fMRI scan only: BMI >25 kg/m2

Exclusion Criteria:
1. Current inhaled nicotine use
2. History of alcohol dependence.
3. Current or past diagnosis of diabetes, or thyroid problems.
4. Hemoglobin A1C >5.7%
5. Taking medications known to influence study measures (including antiglycemic agents, thyroid medications, sleep medications)
6. Active medical or neurologic disorder.
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.)
15. Contraindications for bioelectrical impedance analysis, specifically implanted devices

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 subject that he or she is under no obligation whatsoever to participate, and that if he or she wishes to discontinue their involvement at any time during the study, he or she is free to do so without penalty. Employees will be told that their employment status and evaluation will in no way be affected by their decision to participate or not participate in the study. Students will be advised that their decision to participate will not affect their student status, 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):

We plan to consent approximately 25 participants with the intent of having 15 participants complete all sessions of Aims 1 and 2, and we anticipate having 5 of those participants also complete an fMRI scan. These numbers were determined based on our pilot grant award amount, the sample size we anticipate needing to generate pilot/feasibility data, and the anticipation of ~40% either dropping out or being excluded after enrollment (e.g., unable to determine flavors equally liked and unfamiliar for the conditioning paradigm, hemoglobin A1C outside of 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:

We anticipate screening via the online screening survey approximately 150 potential participants. We anticipate enrolling and screening approximately 25 participants during our Consent and Training Session. We intend to have 15 participants complete all procedures except the fMRI scan. Of those 15 participants, we intend to have 5 participants complete the fMRI scan.

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

Consent and Training Session (flavor tasting/rating, anthropometrics, fingerstick sample for HbA1C): 25 participants
Behavior Sessions 1 and 3 (IV catheter blood draw): 15 participants
Behavior Sessions 3 and 4 (metabolic cart): 15 participants
Post-Test Session without fMRI (flavor and beverage tasting/rating): 15 participants
Post-Test Session with fMRI (flavor and beverage tasting/rating and fMRI scan): 5 participants
Metabolic Chamber Sessions 1 and 2: 15 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 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. 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.

Consent and Training Session (2 hrs) - $40
Behavior Sessions 1-4 (2 ½ hours each) - $50each session, up to $200 total
Post-Test Session (1 hour) - $20
fMRI Scan (2 hours) - $40
Metabolic Chamber Sessions 1-2 (6 hours each)- $120 each session, up to $240 total

Total (with fMRI scan): up to $540
Total (without fMRI scan): up to $500

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 visit 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.

If participants are able to detect the maltodextrin in the triangle test, they will not continue in the study and will be compensated for the training session.

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 while undergoing the study protocol 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. There will be no partial withdrawal.
3. All participant identifying information and data collected up to time of withdrawal will be removed from study database(s).
4. All stored blood samples collected on the participant will be removed from the storage freezer and discarded.
5. 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)*

Food and beverages
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 we will be using to measure gas exchange. These non-invasive procedures are simple and painless, and normal clothes can be worn during the procedures. For the metabolic cart procedure, subjects will be lying back in a chair and a transparent "canopy hood" will be placed over their face and neck. 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 with the hood or chair, they may choose to stop the procedure. For the metabolic chamber procedure, subjects will rest quietly on a hospital bed inside an enclosed chamber for the duration of the test. They will be asked to relax and breathe normally during the entirety of testing. During the testing, study staff will be able to see participants, so should they, at any time during the testing, become uncomfortable, they may choose to stop the procedure.

MRI

The risks associated with fMRI are the same as those with conventional MRI. Movement or heating of metallic implants is a potential risk, therefore subjects will be screened to exclude people with metallic implants, fragments, or pacemakers. Some individuals may experience claustrophobic reactions in the scanner. Any subject experiencing claustrophobia will be removed from the scanner immediately.
The fMRI scanner produces loud noises which could be harmful to the subject. Participants will be required to wear headphones and earplugs 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 pneumatic headphones provided by the scanner manufacturer. These headphones provide some degree of noise reduction. Foam ear plugs with a Noise Reduction Rating of 31 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.

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.

Bioelectrical Impedance Analysis
The risks associated with having body composition measured with bioelectrical impedance analysis are minimal. The likely risks include potential interference with electronic medical device implants, including pacemakers and defibrillators. To minimize these risks, medical device implants are an exclusion criterion for study participation.

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
Indirect calorimetry sessions (Behaviors Sessions 2 and 4, and Metabolic Chamber Sessions) will be performed by trained personnel. Subjects who become uncomfortable during the measurements can withdraw at any time during the procedure.

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

Fingerstick Blood Draw
To reduce the risks associated with performing a fingerstick, research personnel will train participants on the proper technique for performing a fingerstick blood draw and care for the site after the sample is obtained. Participants will also be given separate, single-use blood collecting devices to be used for each blood draw.

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.

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:

No procedures have a known 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 to the individual subject for participation.

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

There is no direct benefit to the individual subject 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 in MRI safety, Human Subjects Protections, and standard laboratory procedures (perceptual ratings, etc.).

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.

Participant personal information and the coded designation will be kept in Ripple™, a secure web application designed for the storing and management of personally identifying 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, including name, participant ID, demographics, and study workflow (e.g., appointments). Participant information managed with Ripple is private and secure. This information 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. Furthermore, Ripple is used only for storing personally identifiable information of participants and is not used to capture other research data (e.g., questionnaires, health records, etc.). This ensures that the personally identifiable information and research data are segregated. 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.

MRI images will be stored on secured FBRI and VT ARC servers.
The behavioral stream of data acquired during experiments will be collected and recorded using an encrypted FBRI laptop, desktop computer, or tablet and then transferred to an FBRI server.

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. The 'key' will be stored in a database which cannot be accessed unless specifically authorized. Deidentified data may be made

available publicly upon publication 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 (such as the Consent Form) will be stored in a separate, locked file cabinet from files containing participant coded identifiers. Study 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 required 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:

This study does not present more than minimal risk.
While the risk involved in this study are minimal, 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. 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.

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 make arrangements to participate outside of work and child-care needed hours.

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.

During the first session (Consent and Training Session), consent for participating in the study will be obtained on-site by research personnel in a quiet, private room or waiting area. 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:

We do not request to waive written consent. See details in 8.0 Procedures Involved for details about consent and training session. In brief, participants will sign the informed consent document in the presence of an investigator in the first study session. They will be emailed a copy of the consent document at least 24 horus before the first session, and the investigator will review the protocol with the participant and answer any questions prior to signing the consent document. Only authorized research personnel will print the consent form from the IRB Protocol Management site to ensure the most recent document is being used. Copies of the signed consent document will be provided to participants.

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:

Consent for this project is attached to this application.

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.

DiFeliceantonio Lab Resources
Dr. DiFeliceantonio's lab is equipped to draw blood using an IV catheter system, analyze blood samples for glucose, perform indirect calorimetry, train participants in the MRI scanner environment, and measure participant's height and weight.
Equipment includes:
Stadiometer - Measurement of height

Bioelectrical Impedance Analysis equipment- Noninvasive measurement of body composition (% body fat)
Hemoglobin A1C analyzer
Scale - Measurement of weight
Parvo-Medics Metabolic Cart – Measurement of gas exchange (O2 consumption, CO2 expiration)
Hemocue Analyzer – Measurement of whole blood glucose (venous)
Centrifuge
MR Simulator (i.e., "mock" MRI scanner; Psychology Science Tools)
Laptop Computer for data collection
Two Tablets for data collection (Lenovo)
Custom gustometer for delivery of liquids in simulated MR environment
3 workstations for research personnel
Laptop with Nutrition Data System for Research (NDSR) software and license

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.

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.