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.

Influence of ultra-processed foods on reward processing and energy intake

PROTOCOL NUMBER:

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

22-253

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VERSION NUMBER/DATE:

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

1.0 Aug 22, 2022
1.1 January 10, 2022

1.2 Feb. 2, 2023

1.3 Feb, 16, 2023

1.4 Nov 10, 2023

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	01-10-23		
2	2-3-23	Add inclusion for those on stable dose of thyroid medications (>6 mo)	Yes
3	2-16-23	Change in physical activity assessment from baseline to the first week of diet period. Remove palatability assessment from diet period. Remove dietary recall from baseline 2	Yes
4	11-10-23	Added two optional 24-hour urine collections to the study procedures, with additional compensation. (ICD, 8.2, 9.1, 9.4, 15.4) Added text to the beginning of the QuestionPro pre-screening survey to re-iterate that we can only include individuals aged 18-25 in the study.	Yes

Table of Contents

1.0 Study Summary

Study Title	Influence of ultra-processed foods on reward processing and energy intake
Study Design	The study will utilize a randomized crossover design, with 2 weeks of controlled feeding followed by measuring changes in reward processing by fMRI. The overall objective of this study is to establish proof-of-concept for an altered reward processing in response to a UPF diet compared to a diet emphasizing MPF in the key developmental period of 18-25.
Primary Objective	To determine the influence of chronic UPF consumption on brain reward response to UPF.
Secondary Objective(s)	To determine the influence of chronic UPF consumption on ad libitum energy intake and food selection. Explore the influence of UPF consumption on executive function, specifically inhibitory control, cognitive flexibility, and working memory, as well as eating in the absence of hunger.
Study Population	Late adolescents/early adults aged 18-25
Sample Size	32
Research Intervention(s)/ Investigational Agent(s)	Participants will be randomly assigned to a 2-week diet emphasizing either UPF (81% total energy) or un/minimally processed foods (0% UPF). Participants will be fed a eucaloric diet (50% carbohydrate, 35% fat, 15% protein) matched for dietary fiber, added sugars, saturated fat, mono- and poly-unsaturated fat, sodium, pre- and probiotics, micronutrients, and overall diet quality, for the duration of the study to avoid potential confounds of weight change and other dietary factors which may influence study outcomes. fMRI will be conducted 4 times where participants will be exposed to UPF milkshakes to assess reward processing. Ad libitum energy intake will be assessed.
Study Duration for Individual Participants	1-2 weeks of screening and baseline testing followed by 2, 14-day feeding periods; followed by 2 days of post-intervention testing and a 4 week washout period in between feeding conditions. The total estimated duration for participants is ~10-12 weeks.
Acronyms and Definitions	UPF: Ultra-processed food MPF: Minimally processed food EI: Energy intake EF: Executive function ED: Eating disorders PA: Physical activity LHS: Labeled Hedonic Scale HEI: Healthy Eating Index TFEQ: Three-Factor Eating Questionnaire mYFAS: modified Yale Food Addiction Scale

	LEC5-Life Events Checklist 5 BIA: Bioelectrical Impedance Analysis DD: Delay Discounting

2.0 Objectives

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

Aim 1: To determine the influence of chronic UPF consumption on brain reward response to UPF.
Aim 2: To determine the influence of chronic UPF consumption on ad libitum energy intake and food selection.
Aim 3: Explore the influence of UPF consumption on executive function, specifically inhibitory control, cognitive flexibility, working memory, and eating in the absence of hunger.

2.2 State the hypotheses to be tested:

We hypothesize blood oxygenation level dependent (BOLD) response in reward associated brain areas (ie. striatum and ventromedial prefrontal cortex) to UPF milkshake will be attenuated following the eucaloric UPF diet compared to the eucaloric MPF diet, and that changes will be associated with increased ad libitum energy intake at the breakfast buffet meal. We further hypothesize that when compared to a eucaloric MPF diet, a eucaloric UPF diet will increase energy intake at an ad libitum breakfast buffet meal and preference for UPF will be increased. We hypothesize that UPF exposure will be associated with reductions in executive function performance compared to MPF exposure, and that eating in the absence of hunger will increase with UPF exposure.

3.0 Background

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

Most individuals with obesity become so before age 35. In the US population, adolescents have the highest ultra- processed food consumption (68% of total energy) and the lowest diet quality. Given that ultra-processed foods represent two-thirds of the energy consumed by adolescents and young adults, research is needed to understand the influence of food processing on eating behaviors, particularly in the late adolescence and

early adulthood life stage. During this time, individuals have increasing independence in food choice and it represents a key developmental period for executive function, processes that affect life-long mental and physical health. Evidence from pre-clinical studies demonstrates rodents maintained on a "cafeteria diet" comprised of ultra-processed ingredients have greater energy intake, weight gain, and alterations in brain circuitry that regulates both energy intake and executive function than those exposed to a standard diet. However, the impact of ultra- processed foods on the brain circuits underlying reward processing, food intake regulation, food choice, and executive function in humans is unknown. Controlled diet interventions are needed to fill this void.

3.2 Describe any relevant preliminary data:

N/A

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:

More than two-thirds of the energy consumed by US adolescents (67%) comes from UPF, and UPF consumption is increasing and UPF exposure is associated with increased ad libitum EI, weight gain and obesity. Furthermore, normal weight individuals aged 18-30 years are likely to become overweight or obese in their next two decades of life; and to date, no experimental studies have investigated the brain response to UPF, or the influence of UPF consumption on ad libitum EI and food selection in adolescents and emerging adults (age 18-25). We will overcome the following limitations of existing research. First, previous studies have not controlled for energy density, which is a major potential confounding factor. Second, the one existing experimental trial on UPF did not include brain imaging measures, or a washout phase, which is needed to address potential carryover effects of high and low UPF diets. Our findings will contribute importantly to an emerging neurobiological understanding of the adverse effects of UPF consumption and how this may contribute to energy overconsumption, weight gain, and obesity risk. This research could have important practice and policy implications. Dietary guidelines for obesity prevention devote minimal attention to processed foods, yet recommendations to limit UPF consumption may be warranted, particularly for adolescents and young adults.

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 endpoint: Assess changes in reward response to UPF-rich milkshake via fMRI before and after each feeding period.
Secondary endpoint: Evaluate energy (kcal) intake and food choice when both UPF and MPF are available immediately following each feeding period.

Other endpoints: eating in the absence of hunger, executive function

*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):

Individuals aged 18-25 years (n=32), will undergo two 14-day controlled feeding conditions in a randomly assigned order (crossover): un/minimally processed diet (0% UPF, “MPF”) or a diet containing 81% energy from UPF (“UPF”). Randomization sequence will be stratified based upon BMI status, sex, and age (18-21y, 22-25y). Diets will be eucaloric and matched for potential confounding factors including macronutrients, fiber, and energy density. A 4-week washout period will occur between dietary conditions. To assess changes in reward processing, brain response to a UPF-rich beverage (“milkshake”) will be assessed via fMRI before and after each feeding period. Executive function will also be assessed before and after each feeding period. Measurements immediately following each feeding period will include ad libitum EI and

food selection containing both UPF and MPF and eating in the absence of hunger post-meal.

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):

Effects on study outcomes will be assessed using mixed effect linear models to estimate treatment differences between diet conditions. Exploratory path analysis with multiple mediators will be utilized to understand mechanisms of change, and estimate effect sizes. Covariates will be included to control for baseline group differences, if any. Standard preprocessing and statistical techniques for fMRI data to look for relationships between neural activity and physiological measures upon chronic exposure to UPF. Standard fMRI processing techniques will also be used to calculate subcutaneous, visceral, and liver fat percentages.

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.*

Recruitment will take place on a continuous basis. Recruitment efforts will include the following strategies: 1) Hard copy flyers. 2) Directly reaching potentially eligible university students through Ads in university-run Listservs 3) Ads in local newspapers. 4) Ads on social media, such as HNFE's and CHBR's Twitter and Facebook accounts. 5) Online story for recruitment avenues such as VT Daily News notices. Interested people will be directed to complete an online survey or contact the Project Director (Dr. Elaina

Marinik, Clinical Studies Coordinator for HNFE) or Blacksburg Study Coordinator, who will schedule an initial phone screening to determine eligibility. Participants will be men and women recruited from in and around Blacksburg, VA and the New River Valley region who meet study eligibility criteria. Study procedures for the assigned diets will primarily occur on the Virginia Tech Blacksburg Campus (Wallace Hall and Garvin Innovation Building). The study procedures for fMRI measurements will all take place at the Fralin Biomedical Research Institute (FBRI) at VTC campus in Roanoke, VA. Training, conditioning, and post-testing sessions will occur in the Center for Health Behavior Research (CHBR) at the FBRI. The MRI scans will take place in the Human Neuroimaging Lab (HNL) at the FBRI.

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

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

- *Drug/Device Handling: If the research involves drugs or devices, describe your plans to store, handle, and administer the drugs or devices so that they will be used only on subjects, and only by authorized investigators.*
- *Describe whether any of the following will be used: microwaves, X-rays, DEXA scans, general anesthesia, or sedation*
- *If control of the drugs or devices used in this protocol will be accomplished by following an established, approved organizational SOP (e.g., Research Pharmacy SOP for the Control of Investigational Drugs, etc.), please reference the SOP in this section.*

Device Handling:
This research 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:

Physical activity levels will be assessed using an accelerometer (wGT3X-BT, Actigraph Inc.), which will be worn like a watch on the wrist for 4 days during each diet phase. This device is cleared by the FDA and will be used in a manner consistent with its approved use.

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

Body composition will be assessed using Bioelectrical Impedance Analysis (BIA), which is similar to a scale. It will be used in a manner consistent with its intended and approved use.

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:

Individuals aged 18-25 years (n=32), will undergo two 14-day controlled feeding conditions in a randomly assigned order (crossover): un/minimally processed diet (0% UPF, “MPF”) or a diet containing 81% energy from UPF (“UPF”). Randomization sequence will be stratified based upon BMI status, sex, and age (18-21y, 22-25y). Diets will be eucaloric and matched for potential confounding factors including macronutrients, fiber, and energy density. A 4-week washout period will occur between dietary conditions. To assess changes in reward processing, brain response to a UPF-rich beverage (“milkshake”) will be assessed via fMRI before and after each feeding period. Subcutaneous, visceral, and liver fat percentages will also be assessed via fMRI before and after each feeding period. Discounting Rate will be measured by computerized money task. Measurements immediately following each feeding period will include: ad libitum EI and food selection containing both UPF and MPF, eating in the absence of hunger post-meal, and executive function.

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.*

Please see "Schedule of Study Activities/Assessments" on a following page, for a graphic representation of study procedures.

Eligibility Screening Procedures: Participants will first complete an online or phone pre-screening survey. If they are potentially eligible after completing the screening survey, they will be sent the informed consent document via email at least 24 hours prior to their initial screening and they will have an initial in-person screening visit scheduled.

Screening visit 1 (229 Wallace Hall; ~1-1.5 hours): During the first in-person screening visit, a researcher will go over the informed consent document with the participant prior to collection of any data. If the participant gives consent, then the first screening visit will continue. Baseline screening measures will include: Health history form, screening forms for eating behaviors (mYFAS, TFEQ), LEC5, habitual dietary intake (in person 24-hour recall), , height, and weight. Review protocol with the participant for two 24 hour recalls which will be done by phone.

Screening visit 2 (1 Riverside Circle-CHBR Suite; ~ 1 hour): The second laboratory screening visit will consist of a mock fMRI scan to improve data quality and ensure

participants are eligible and willing to complete the fMRI measurement. Participants will fill our clincard forms and receive clincard.

Baseline Measurement 1, Diet Period 1 (229 Wallace Hall; ~1-1.5 hours): If participants are eligible and willing they will come in and undergo measurement of body composition (BIA), hip and waist measurements, and executive function.

Baseline Measurement 2, diet Period 2 (2 Riverside Circle-Human Neuroimaging Lab; ~2 hours): The second baseline measurement will consist of participants undergoing fMRI tasks with a dairy based milkshake and an abdominal fMRI. Participants will also complete a computerized Delay Discounting measure. Participants will then be randomly assigned to their first diet condition.

Two-week diet period 1 (12 daily visits [Mon-Sat], 334 Wallace Hall; ~30 min/day, 6 hours total): Following the eligibility screening and baseline measurements, participants will come into the lab each morning in the fasted state, be weighed (to insure weight stability), eat their breakfast in the dining laboratory, and be provided with a cooler of remaining food for the day.On the first Saturday of diet, participants will receive the Actigraph and have them until Thursday, when they should return it.. During the second week of the diet, the following measurements will be made: On day 13, executive functioning, body composition (BIA), and waist and hip measurements will be assessed. On day 14, meal palatability will be assessed using the LHS after their supervised breakfast meal, then they will travel to Roanoke for fMRI measurements.
Optional component: Participants may provide a 24-hour urine sample during this diet period, if they are willing to do so.

Day 14 fMRI 1 (2 Riverside Circle-Human Neuroimaging Lab; ~3 hours): After meal palatability assessment, the participant will travel to Roanoke to undergo fMRI with a dairy based milkshake after diet period to assess changes in brain response after exposure to the diet condition. Abdominal fMRI will be repeated to assess changes in subcutaneous, visceral, and liver fat. Computerized delay discounting task will be repeated after the diet period to assess any changes in discount rate. fMRI assessments will be performed ~ 3 hours after the supervised breakfast meal.

Ad libitum Buffet Meal: (334 Wallace Hall; ~ 1 hour): On day 15, the participant will rate internal state, engage in an ad libitum energy intake and food selection assessment. Visual Analog Scales (VAS) will be completed at time 0 (baseline) and time 30 (30 minutes after exposure to the buffet meal). Time spent eating will be covertly recorded (meal start/stop time). Kcal and grams of food consumed will be covertly recorded, to calculate meal eating rate (g/min, kcal/min). Foods selected will also be covertly recorded. Eating in the absence of hunger will be assessed immediately after the buffet meal. Four snacks items will be provided and participants will be asked to take a bite of each one and rate its palatability using the LHS, and then be left alone to eat the remainder of the snacks or look at the magazines while they wait for the next part of the study. Participants will be left to consume the snacks or relax for 15 minutes.

Eating in the Absence of Hunger Measurement (Take Home): Participants will be given a box of snacks to assess eating in the absence of hunger outside the laboratory environment. Snack boxes will be returned to study staff the next morning. Staff will document the amount and type of snacks consumed.

4 week washout period: Participants will be asked to resume their usual diet. In the final week of the washout (week 4), assessments of dietary intake, PA, EF, and fMRI will be completed in two baseline visits for the second feeding period (listed below). These will serve as the baseline assessments for the second feeding period. For females, washout duration may be extended to 6 weeks if needed, in order to schedule EF, fMRI, and ad libitum buffet meal post- testing in the same menstrual cycle phase.

Baseline Measurement 1, Diet Period 2 (229 Wallace Hall; ~1-1.5 hours): Participants will undergo executive function, BIA with hip and waist measurements,

Baseline Measurement 2, diet Period 2 (2 Riverside Circle-Human Neuroimaging Lab; ~3 hours): The second baseline measurement will consist of participants undergoing fMRI tasks with a dairy based milkshake. Abdominal fMRI will be completed to assess changes in subcutaneous, visceral, and liver fat. Computerized delay discounting task will be completed.

Participants will then undergo Feeding Period 2, following the same procedures used in feeding period 1. A BIA with hip and waist measurements will be performed on day 13, when the EF measures are completed.
Optional component: Participants may provide a 24-hour urine sample during this diet period, if they are willing to do so.

The total number of visits will be ~34; the exact number of visits may vary slightly due to scheduling of research staff and participants. Total number of hours for participants will be ~33-35.

Schedule of Study Activities/Assessments: Young Adults Eating Habits

Procedures	Eligibility Pre-Screening	In-Person Screening		Baseline Measures 1 (wk prior to diet)		14-d Diet Period 1 (12 visits*)		4-wk Washout **	Baseline Measures 2**		14-d Diet Period 2 (12 visits*)	
	Online/ Phone	Day 1	Day 2	Day 1	Day 2	Wk 1	Wk 2		Day 1	Day 2	Wk 1	Wk 2
Pre-Screening Survey QuestionPro	x											
ICD sent via email	x											
ICD reviewed, signed		x										
Health History Form Mens, cycle calendar (paper form)		x										
mYFAS		x										
TFEQ		x										
Life Events Checklist		X										
IPAQ (short)		X										
24-hr diet recall *2nd & 3rd via phone		x										
Height, Weight		x										
Weight (daily during diet periods) Start diet-Fridays (women: early follicular phase)		x		x		X d. 1 Fri	x		x		x	x
Mock fMRI scan (Roanoke)			x									
Assigned to Diet Condition (sequence) Roanoke mock fMRI			x									
ClinCard (fMRI visits) Roanoke			x				x					x
BIA with Waist:Hip ratio				X M/T/W			X d. 13 Wed		X M/T/W			x d. 13 Wed
Executive Function				X M/T/W			X d. 13 Wed		X M/T/W			X d. 13 Wed
PA (4 d. Actigraph)						X Sa-Th					X Sa-Th	
fMRI with milkshake, 3 hrs post-bfast*** with Liver Fat Roanoke					X Th		x d. 14 Th			X Th		X d. 14 Th
DD task Roanoke					X Th		x d. 14 Th			X Th		X d. 14 Th
Ad Lib Buffet Meal with VAS at min 0 & 30							X d. 15 Fri					X d. 15 Fri
EIAH (lab), w/ LHS (15 min)							X d. 15 Fri					X d. 15 Fri
EIAH (home) Snackbox, LHS, VAS Return Saturday (& pay via ClinCard)							X d. 15 Fri					X d. 15 Fri
DeBrief & Final Report to Participant												X

*During the controlled diet periods, participants will come to the Dining Lab daily for breakfast Monday-Saturday. On Sat, they will receive food for Sunday. Body weight will be measured at each visit to insure weight stability.

**Baseline 2 measures will be done during the final week of washout (week 4)-can extend washout for women if needed (begin diet in early follicular phase of menstrual cycle or anytime for women with IUDs.
***Baseline fMRIs will be done after the participants usual breakfast since they are not on the controlled diet for the baseline test.

Detailed description of measures:

Medical History: Subjects will be asked to complete a medical history form, which will be used to screen for health problems (heart disease, stroke, respiratory disease, endocrine or metabolic disease, hematological/oncological disease, medication usage, food allergies and aversions) that would preclude participation. Usual physical activity habits will also be reported.

24-hour dietary recall: Participants will be asked to describe all foods and beverages consumed in the previous 24 hours. NOTE: a birthdate is required by this software when dietary data are entered, in order to generate a table of the participant's reported intake compared to age- and sex-based Dietary Reference Intake recommended intake values.

Mock fMRI training: "mock" MRI training session will be completed in the CHBR during which subjects will learn to swallow small amounts of liquids while lying down (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. Subjects who have difficulty with supine swallowing or who express discomfort in the simulated fMRI environment will not continue in the study. At this time they will also be trained on the use of the LHS. This involves rating imagined sensations on the scale.

Body Mass and Composition: Body weight will be measured on a digital scale accurate to +/-0.01 kg. Height will be measured with a stadiometer. Percent body fat and fat-free mass will be measured in all subjects using BIA. This is a non-invasive technique that uses a device similar to a scale. A weak electric current flows through the body from two points, and the rate at which the current travels through the body can be used to estimate lean and adipose tissue mass. The current is not detectable to the individual undergoing the procedure.

Hip and Waist Circumference: Participants hip and waist circumference will be measured following the WHO guidelines at the time of the BIA measurement. The procedure should be done with stretch-resistant tape that is wrapped snugly around the subject, but not to the point that the tape is constricting, at a level parallel to the floor. The participant should be standing upright during the measurement, with arms relaxed at the side, feet evenly spread apart and body weight evenly distributed. Waist measurement: in the midpoint between the top of the iliac crest and the lower margin of the last palpable rib in the mid-axillary line. Measurement of the waist circumference should be done at the end of several consecutive natural breaths. Hip measurement: at the largest circumference of the buttocks.

fMRI procedures: During this session, 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. Using a body coil, structural images are acquired using the Dixon-VIBE 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/or 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 water .
After completion of the milkshake task, the participant will have their head-coil switched for the body coil, and begin the abdominal fMRI scan. This is done by a standard Dixon sequence with short, automated breath holds.
Due to the nature of fMRI and quality control, participants may be asked to come back to Roanoke and repeat the fMRI measurements within 2-5 days after completion of the fMRI.

Delay Discounting: A computerized 5-trial adjusting delay discounting task will be used to understand valuation of reward upon increasing time to receipt. This will give the participants associated discount rate (k) and effective delay 50% (ED50).

Eating behavior: Questionnaires (in attachments) assessing eating habits will be collected on a computer.

Physical activity: Participants will wear a wrist-worn accelerometer (wGT3X-BT, Actigraph Inc.) for 4 full days (first Saturday of Diet period until Thursday); this is similar to a watch.

Executive function: We intend to assess working memory, cognitive flexibility, and inhibitory control using the Corsi Block-tapping test, a variant on the Flanker task, and a Go/no-go task. The Corsi Block-Tapping test takes approximately 10 minutes to

complete, and requires participants to watch a series of squares light up in a particular sequence, and then click the blocks in the forward or reverse order that they were highlighted. The Flanker task takes approximately 8 minutes to complete and requires participants to observe a set of arrows in which the middle arrow is directed in either the same or a different direction to the others in the set, and to indicate which direction the middle arrow is pointing. The Go/No-Go Test takes approximately 8 minutes to complete, and requires the participant to press a button whenever certain items or letters are presented on screen, but to withhold a response when one particular item is presented. These tasks have been widely used with participants across the lifespan and contain simple graphics. Previous studies have linked these and similar tasks to dietary choices (e.g. Batterink, Yokum, & Stice, 2010) and thus they are appropriate for examining the links between cognition and eating behaviors. Participants will receive brief instruction on each of the tasks from a research assistant prior to completing them.

Buffet Meal. Participants will arrive at the laboratory in the fasted state for an ad libitum breakfast buffet meal. Using an approach similar to our previous studies the buffet meal will contain a variety of food items that vary in macronutrient content and in their degree of processing (UPF, MPF). The meal will consist of typical breakfast items (e.g., oatmeal, Lucky Charms, Pop Tarts, bananas, 100% juice, Sunny Delight) in excess of what would normally be consumed. Participants will self-select foods over a 30-minute period. Meal eating rate (g/min, kcal/min) will be determined using meal start and stop times 4. Food items will be weighed (±0.1 grams) prior to and after the completion of the meal to determine the amount consumed (g, kcal), calculated using NDSR (2022) nutritional analysis software. Food selection will be determined by the number of items, grams and energy consumed from UPF vs MPF. To assess sensations of hunger and fullness, Visual Analog Scales (VAS) will be completed at prior to (min. 0), and following the buffet meal (min. 30). Following the buffet meal and the 30-minute VAS, participants will be assessed for eating in the absence of hunger.

Eating in the absence of hunger: Participants will move to a separate room where an ample amount of 4 pre-weighed snack items (2 UPF, 2 MPF) and magazines are available. Using an approach consistent with previous research studying this outcome, participants will be instructed to take 2 bites of each snack item and rate its palatability on the LHS, and to eat the remainder of the snacks or look at the magazines while they wait for the next part of the study. Participants will be left to consume the snacks or relax for 15 minutes.

24-hour urine collection (optional): Willing participants will be provided with two containers and instructions to collect all urine for a 24-hour period during each diet period. Urinary excretion of nutrients such as sodium will be measured, to evaluate compliance to the controlled diet. Additional urine assessment may be done related to kidney health or other outcomes.

8.3 Describe:

- *Procedures or safeguards intended to <u>reduce the probability and magnitude of risks</u>. (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*

There is risk of an allergic reaction to study provided meals if a participant has an unknown food allergy (those with known food allergies will be excluded from participation). There is also a risk of food-borne illness due to improper food preparation, storage, and handling on the part of both research staff and participants.

Procedures to minimize risk:
Informed Consent Process. Potential participants will be informed about all study procedures, outline the risks and benefits, explain the commitments of the participants and investigators, and answer all questions potential participants may have. Prior to participation in the study, participants will review and sign an informed consent document detailing the previously mentioned information. A copy of the consent document will be provided for the participants to take with them.

Safety of Proposed Research Diets. We are providing participants with an amount of food required to maintain their body weight. As such, there should be no weight gain. Research dietitians and technicians will be monitoring the body weight of participants on a daily basis to insure weight stability. In the event that energy requirements are overestimated, participants will be instructed to return un-eaten food to our metabolic kitchen. In the event of weight loss, controlled diets will be adjusted to provide additional calories. While there is a risk of food poisoning, food allergies, and gastrointestinal discomfort with research diet administration, these will be minimized by: a) having all diets prepared and packaged in our metabolic kitchen by ServSafe-certified research staff, b) training research staff on proper food handling, preparation, and storage etc. protocols consistent with ServSafe food safety guidelines, c) to minimize risk of participants engaging in improper food safety practices, food will be provided in temperature-controlled coolers and information regarding proper storage and reheating of foods will be provided. The Metabolic Kitchen is located on the Virginia Tech campus, directed by Brenda Davy, PhD, RDN who has over 20 years of experience conducting clinical research, designing and preparing research diets, and conducting a detailed interview

with all participants prior to participation to screen for known food allergies, sensitivities, or aversions. List of foods to be used on the controlled diets will be reviewed with participants prior to enrollment.

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.

Participant privacy and confidentiality of data will be maintained by 1) training research staff; 2) limiting access to participant information to key research study personnel; 3) assigning participants a non-identifiable study code; 4) storing study codes, participant information and contact information separately from raw data; and 5) securing raw data and electronic data in locked file cabinets and on password protected computers, respectively.

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:

We will collect the following:
Health history, demographic information, eating behaviors and life events by questionnaire
Waist and Hip Circumference
Body weight, height and composition (by BIA)
Habitual food intake by dietary recalls
Habitual physical activity by accelerometry
MRI safety information will be collected via paper form (attached).
Perceptual ratings (LHS, VAS) will be collected using a computer

Executive function measures will be taken using standard software
Food intake will be assessed by weighing food before and after consumption
Brain images will be acquired via MRI scans
Abdominal and Liver images will be acquired via MRI scans
Delay Discounting measures will be taken using RedCap

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

N/A

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)*

This research involves no more than minimal risk to participants. We would like to refer to the study in recruitment materials as "Young Adult Eating Habit (YAEH) study" rather than its full title. We are concerned if we draw attention to our interest in studying how ultra-processed foods impact eating behaviors including calorie intake and food selection, participants may change their eating behavior in the ad libitum buffet meal and snack assessment, which will affect our study measurements and results. This will preclude us from achieving the second aim of our project. We will not refer to the ad libitum buffet meal as such, but rather let participants know that they will be provided with breakfast in our lab. They will not be aware that we are evaluating their energy intake and food selection at that meal. This will not adversely affect the rights and welfare of the participants. Participants will be provided with a debriefing document that explains the incomplete disclosure. They will be given the opportunity to withdraw these data from the study. They will be asked to sign this document indicating their agreement to be in the study.

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:

N/A

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:

Data will be coded using a 6 character number. Participant IDs will be created sequentially starting at YAEH01 . The key linking the participant name or identifiable information and the code will be stored on an encrypted server managed by the FBRI and HNFE 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. De-identified participant information will be kept indefinitely or at the written request of the participant. De-identified data and urine samples may be shared with others outside of the research team without reconsent.

Dietary recall data will be recorded using a standardized 24-hour recall form. Forms will be stored in a locked cabinet in Wallace Hall. Data will be analyzed using NDS-R on the password-protected desktop computer located in the PI Davy's locked laboratory in Wallace Hall (rm 334).
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.

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

N/A

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:

N/A

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 6 character number. Participant IDs will be created sequentially starting at YAEH01. The key linking the participant name or identifiable information and the code will be stored on an encrypted server managed by the FBRI and HNFE 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. De-identifiedIdentifiable participant information will be kept indefinitely destroyed 5 years after publication or at the written request of the participant. De-identified data and urine samples may be shared with others outside of the research team without reconsent.

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:

Participants will receive information about:
- dietary intake analysis
- height, weight, body composition
- copy of brain and abdomen MRI if desired

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.*

The total estimated duration for individual participants is ~10-12 weeks involving 1-2 weeks of screening and baseline testing followed by 2, 14-day feeding periods; followed by 2 days of post-intervention testing and a 4 week washout period in between feeding conditions. The number of study visits will be ~34 and the total number of hours required for participation will be ~33-35 hours.
Approximately 1 year will be required to recruit and enroll participants. Approximately 1.5 - 2 years will be required to complete the study data collection and analysis. If recruitment goals are not met within 2 years, a 1-year no-cost extension will be requested from the NIH if study funds are available to continue recruitment and complete data collection/analysis.

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:

Inclusion Criteria:
Age 18-25 years
Weight stable (+5 lbs) for previous 6 months
No plans to gain/lose weight or change physical activity level
Willing to pick up food daily and consume foods provided for two 14-day periods
Verbal and written informed consent
Unrestrained eater (TFEQ cognitive restraint score <11)
No reported history of eating disorders
Usual UPF intake +/-10% of US adolescent average of 68% total energy
Sedentary to recreationally active
*Individuals on a stable dose of thyroid medication (>6 mo) are eligible to participate, provided that they have been weight stable for the previous 6 months. Thyroid medication changes can cause weight instability.

Exclusion criteria:

BMI >30 kg/m2
Endocrine disorders* or other major chronic disease (e.g., type 2 diabetes, hypothyroidism, hypertension)
Pregnant or plans to become pregnant
Food allergies or aversions

Claustrophobia
History of head injury with loss of consciousness for more than 10 minutes
Contraindications to MRI: individuals with pacemaker, aneurysm clips, neurostimulators, cochlear implants, metal in eyes, steel worker, or other implants.
Eligibility Screening Procedures: Participants will first complete an online or phone pre-screening survey. If they are potentially eligible after completing the screening survey, they will be sent the informed consent document via email at least 24 hours prior to their initial screening and they will have an initial in-person screening visit scheduled.

Screening visit 1 (229 Wallace Hall): During the first in-person screening visit, a researcher will go over the informed consent document with the participant prior to collection of any data. If the participant gives consent, then the first screening visit will continue. Baseline screening measures will include: Health history form, screening forms for eating behavior (mYFAS, TFEQ), life events checklist (LEC5), habitual dietary intake (in person 24-hour recall), physical activity assessment questionnaire, height, and weight. Review protocol with the participant for two 24 hour recalls which will be done by phone.

Screening visit 2 (1 Riverside Circle-CHBR Suite): The second laboratory screening visit will consist of a mock fMRI scan to improve data quality and ensure participants are eligible and willing to complete the fMRI measurement. Participants will fill our clincard forms and receive clincard.

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:
Age 18-25 years
Weight stable (+5 lbs) for previous 6 months
No plans to gain/lose weight or change physical activity level
Willing to pick up food daily and consume foods provided for two 14-day periods
Verbal and written informed consent
Unrestrained eater (TFEQ cognitive restraint score <11)
No reported history of eating disorders and EAT-26 score <20
Usual UPF intake +/-10% of US adolescent average of 68% total energy
Sedentary to recreationally active
*Individuals on a stable dose of thyroid medication (>6 mo) are eligible to participate.

Exclusion criteria:
BMI >30 kg/m2

Endocrine disorders* or other major chronic disease (e.g., type 2 diabetes, hypothyroidism, hypertension)
Pregnant or plans to become pregnant
Food allergies or aversions
Claustrophobia
History of head injury with loss of consciousness for more than 10 minutes
Contraindications to MRI: individuals with pacemaker, aneurysm clips, neurostimulators, cochlear implants, metal in eyes, steel worker, or other implants.

No geographical criteria as long as participants are willing to pick up study food daily on the Virginia Tech Blacksburg campus and complete baseline and post study measurements at the Roanoke Campus.

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*

No vulnerable populations listed above will be included.

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.*

N/A

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):

Planned enrollment is 32 participants.
Briefly, for the primary aim, a power analysis based on effect sizes from the literature was conducted using the pwr toolbox in R, from which a sample size of 26 was determined. For the secondary aim, effect size was calculated using effsize and a power analysis was conducted using pwr, which determined 13 participants were needed. Therefore, we will recruit 33 participants to account for 20% attrition, for a final sample of 26.

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:

N/A

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:

Based upon previous experience implementing studies with similar protocols, we expect to screen 100-150 subjects, for a final enrollment of 32.

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

N/A

15.0 Recruitment Methods

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

Recruitment will take place on a continuous basis. Recruitment efforts will include the following strategies: 1) Hard copy flyers. 2) Directly reaching potentially eligible university students through Ads in university-run Listservs 3) Ads in local newspapers. 4) Ads on social media, such as HNFE's and CHBR's Twitter and Facebook accounts. 5) Online story for recruitment avenues such as VT Daily News notices. Interested people will be directed to complete an online survey or contact the Project Director (Dr. Elaina Marinik, Clinical Studies Coordinator for HNFE) or Roanoke Study Coordinator, who will schedule an initial phone screening to determine eligibility.

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):

General population, Virginia Tech community, local community

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

Responses on the online or phone pre-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.*

All recruitment advertisements and text for email recruitment are uploaded to Protocol Management.

Participants will receive a total of $400 for completing the study as follows:
Upon completion of eligibility and screening visits, $20.
Upon completion of diet period 1 , $190.
Upon completion of Debrief and return of EAH take home box (end of feeding period 2), $190.
Upon completion of two optional 24-hour urine collections, $25 per collection.

Payments will be made using the ClinCard system.

Incentives for Completion:
A 3D printed brain will be used as an incentive for full completion of study and participants will be awarded their 3D printed version of their own brain as a completion bonus.

16.0 Withdrawal of Subjects

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

Non-compliance with study procedures, including consumption of the controlled diets. 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.

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

Participants will be provided with an informational packet containing a summary of their body weight and composition and usual dietary intake. The packet will contain reference values, if available, and current recommendations from major health or federal organizations such as the Dietary Guidelines for Americans 2020-2025.

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

The following steps will be taken in the event a participant withdraws from the study and informs us they want their information destroyed:
1.The participant database will be updated that the participant withdrew (for all withdrawals).
2.Participant will be compensated for their time and sessions completed up to time of withdrawal. There will be no partial withdrawal.

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 Consumption: There is risk of an allergic reaction to study provided meals if a participant has an unknown food allergy. There is also a risk of food-borne illness due to improper food preparation, storage, and handling on the part of both research staff and participants.

MRI data collection: 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.

Milkshake stimuli: All components of the beverage 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.

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.)

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.

Informed Consent Process: Prior to participation in the study, participants will review and sign an informed consent document detailing the previously mentioned information on risks. A copy of the consent document will be provided for participants to take with them.

Safety of Proposed Research Diet:. We are providing participants with an amount of food required to maintain their body weight. As such, there should be no weight gain. Research dietitians and dietetic technicians will be monitoring the body weight of all subjects on a daily basis. In the event that energy requirements are overestimated, participants will be instructed to return un-eaten food to our metabolic kitchen. In the event of weight loss or reports of substantial hunger, diets will be adjusted to provide additional calories. Safety will be further ensured by: a) only recruiting individuals without extreme dietary patterns; b) minimizing the duration of the intervention period as much as possible while also ensuring sufficient time to observe changes in our primary outcome measures; and c) only recruiting healthy individuals. While there is a risk of food poisoning, food allergies, and gastrointestinal discomfort with research diet administration, these will be minimized by: a) having all diets prepared and packaged in our metabolic kitchen by ServSafe-certified staff. The Metabolic Kitchen is located on the Virginia Tech campus, directed by Brenda Davy, PhD, RD who has over 20 years of experience conducting clinical research, designing and preparing research diets; b) designing diets based on participant's food preferences to the extent possible; and c) conducting a detailed interview with all subjects before starting the study to screen for known food allergies, sensitivities, or aversions. List of foods to be used on the controlled diets will be reviewed with participants prior to enrollment. Risk of food-borne illness will be minimized by training research staff on proper food handling, preparation, and storage etc. protocols consistent with ServSafe food safety guidelines. To minimize risk of participants engaging in improper food safety practices, food will be provided in temperature-controlled coolers and information regarding proper storage and reheating of foods will be provided.

The intervention and measurement protocols pose minimal risk to participants. The data safety monitoring plan (DSMP) for this study focuses on close monitoring by the MPI and the safety officer, along with prompt reporting of excessive adverse events and any serious adverse events (AEs) to the NIH and to the Institutional Review Board at Virginia

Tech (VT IRB). All serious AEs will be reported by the PI within 48 hours of occurrence to the VT IRB. Safety reports will be sent to the MPI and MD safety officer. The MPI will be responsible for assembling the data and producing these reports as well as assuring that all parties obtain copies of these reports. Reports will be submitted annually to the VT IRB for review. The frequency of data review for this study differs according to the type of data, the availability of data collected, and the perceived level of risk. Participants will be queried at each laboratory visit when meals are picked up as to any side effects or changes in health status that they experience, over the two 2-week controlled feeding interventions.

Any reported side effects will be discussed immediately with the MPI and the safety officer. The safety officer will determine if medical intervention or other actions are warranted. Participants will be referred to their primary care physicians if there are incidental findings.

Data type	Frequency of reviewing reports
Subject accrual (adherence to the protocol regarding demographics, inclusion/exclusion)	Semi-annually
Adverse event rates	Semi-annually
Compliance to treatment	Semi-annually
Out of range laboratory data	Semi-annually
Stopping rules report regarding statistical power implications of drop outs and missing data	Semi-annually
Side effects of controlled diet, study procedures	Daily, when meals are picked up

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:

N/A

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

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:

The study does not have any potential benefits to participants. However, it may contribute to participants' knowledge regarding their health.

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

There are no anticipated direct benefits to participants.

19.0 Data Management and Confidentiality

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

Nutrient composition of the controlled diets will be verified using food ingredient labels and standard food composition tables.

A detailed Manual of Procedures (MOP) will be developed, to improve intervention fidelity and replicability. All study staff will be trained in study procedures prior to implementation. The project director will conduct random audits of foods provided to participants to verify accuracy of controlled diet provision.
All data will be collected and analyzed by trained research personnel. They will be trained via MRI safety training, Human Subjects Protections, and vigorous training in standard laboratory procedures (perceptual testing, 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*

N/A

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.:

Data will be coded using a 6 character number. Participant IDs will be created sequentially starting at YAEH01. The key linking the participant name or identifiable information and the code will be stored on an encrypted server managed by the FBRI and HNFE 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. De-identifiedIdentifiable participant information will be kept indefinitely or at the written request of the participant. De-identified data may be shared with others outside of the research team without reconsent.

Dietary recall data will be recorded using a standardized 24-hour recall form. Forms will be stored in a locked cabinet in Wallace Hall. Data will be analyzed using NDS-R on the password-protected desktop computer located in PI Davy's locked laboratory in Wallace Hall (rm 334).

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.

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):

N/A

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?*

All study data will be stored on the PI's, password-protected laboratory computer and secured servers (FBRI and HNFE) until at least 5 years after the last publication or presentation. All participant study data will remain de-identified and stored in code format.

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 data required for the study will be collected - to determine eligibility, collect data required as part of the study, or to communicate with participants about issues such as scheduling study visits. Data will not be shared with researchers or research staff not involved with this study. Participant privacy and confidentiality of data will be maintained by 1) training research staff; 2) limiting access to participant information to key research study personnel; 3) assigning subjects a non-identifiable study code; 4) storing study codes, subject information and contact information separately from raw data; and 5) securing raw data and electronic data in locked file cabinets and on a password protected computers and secure servers, respectively.

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):

All study measurements (height, weight) will be done in a private clinical testing room or area, with only study staff present. Participants will be given the option to receive results of body measurements, but will also be given the option to be weighed blindly.

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:

N/A

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)*

N/A

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

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

21.1 Describe:

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

The data safety monitoring plan (DSMP) for this study focuses on close monitoring by the multiple principal investigators (MPI) and study medical director, along with prompt reporting of excessive adverse events and any serious adverse events (AEs) to the NIH and to the IRB at Virginia Tech (VT IRB). All serious AEs will be reported by the MPI within 48 hours of occurrence to the VT IRB.

Safety reports will be sent to the MPI and study MD. The Project Coordinator will be responsible for assembling the data and producing these reports as well as assuring that all parties obtain copies of these reports. Reports will be submitted annually to the VT IRB for review.

The frequency of data review for this study differs according to the type of data, the availability of data collected, and the perceived level of risk (see table below). Participants will be queried at each laboratory visit when meals are picked up as to any

side effects or changes in health status that they experience, over the two, 2-week controlled feeding periods.

Data type	Frequency of reviewing reports
Subject accrual (adherence to the protocol regarding demographics, inclusion/exclusion)	Semi-annually
Adverse event rates	Semi-annually
Compliance to dietary treatment	Semi-annually
Stopping rules report regarding statistical power implications of drop outs and missing data	Semi-annually
Side effects of controlled diet, study procedures	Daily, when meals are picked up

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:

No compensation is available for research-related injury.

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:

N/A

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:

Taking part in this research may lead to added costs such as transportation to the study location to pick up food and to undergo fMRI measurements. Parking on campus will be available at no cost to participants. The SmartWay Express Bus is available for free to university students and a small fee to non-students that connects the two campuses where

all measurements take place. There are bus stops at each location in close proximity to the MPI's labs. We do not anticipate any additional uncompensated costs.

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*

Interested participants will be given a minimum of 24 hours to review the informed consent document before the appointment where the informed consent process will take place. Informed consent documents will be e-mailed (or mailed or picked-up in person, if applicable) by a potential participant prior to the appointment. The informed consent process will be conducted in the PI's laboratories, either 229 or 334 Wallace Hall, by either the study coordinator, a trained graduate student, or a PI. We will review the consent form, explain the study procedures, outline the risks and benefits, explain the commitments of the participants and investigators, and answer all questions potential participants may have. Following that session, individuals who wish to participate will

sign an informed consent document detailing the previously mentioned information. A copy of the consent document will be provided for participants to take with them.

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.*

N/A

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).*

The study name on recruitment and consent form materials will be Young Adults Eating Habits (YAEH) so as to keep participants somewhat naive about the exact focus of our study, which is the impact of ultra-processed food exposure on energy (kcal) intake and food selection. There will be an alteration of the consent process for this study. Participants will receive incomplete information about the purpose behind the ad libitum food intake measure at the buffet meal and the eating in the absence of hunger measure collected following the buffet meal. Participants will not be informed that their food consumption will be measured during both of these portions of the study; this will be done covertly by research staff when the participant leaves the room. This alteration is necessary because the ad libitum food intake aim could not be practically carried out without withholding this information. Knowing the purpose of the study could impact participants' food selection and intake at the buffet meal, which will impact study results.
Following their participation in the study, participants will be given additional information about the measures associated with the buffet meal and snacks. This

debriefing process will include providing them with a debriefing document, and they will be given the opportunity to withdraw these data from the study. They will be asked to sign this document to indicate that they agreed to have their data used. Either the MPI or the study coordinator will provide disclosure on the true purpose of this study visit.

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.*

N/A

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.*

N/A

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:

Participants will sign the informed consent and the signed informed consent document will be stored in a locked filing cabinet. The PI or study coordinator obtaining consent will also sign the informed consent after the subject has provided their signature.

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):

N/A

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:

N/A

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).*

This research project is supported through a 2-year grant from the National Institute of Aging at the NIH. The PIs, Davy and DiFeliceantonio, have devoted 10% effort to this grant/research study. All co-investigators are supported at 5% effort for this research, for

two years. Graduate student support is also available for this trial. All graduate students, undergraduate students, Co-I's and study staff will be trained in all procedures and complete protection of human research participant training. Study staff will be trained in all procedures prior to study onset, and a Manual of Procedures will be available for staff training and reference.

Our research group has successfully completed numerous large-scale NIH-funded intervention trials in this region, including those using controlled feeding procedures. The MPI have experience with recruiting and retaining uniquely challenging study populations, such as young adults, and young children and adolescents (n=337 recruited in 1.5 years, 49% male, 90% retention; R21 HD078636). Given our experiences, enrolling 32 individuals in 1-1.5 years is feasible. If recruitment targets are not met and support is available, recruitment will continue and a no-cost extension requested from the NIH.

Facilities:
Clinical/Laboratory. The Laboratory for Eating Behaviors and Weight Management (Director: B. Davy) is located in Wallace Hall on the Virginia Tech campus. This clinical research space encompasses ~900 sq ft research Dining Laboratory area with ~600 sq ft Metabolic Kitchen, a research dietitian computer workstation, three reach-in freezers, a refrigerator for storing study foods and a large commercial True brand reach-in refrigerator for storing participant meals to be consumed off-site, dry storage areas, two stoves, three sinks, a dishwasher, microwave, commercial toasters and coffee makers, four Sartorius Practum scales for weighing study foods, and a washer/dryer. The Dining Laboratory area contains five tables and 15 chairs for study participant seating/meal consumption. The Metabolic Kitchen has the capability to feed 10-12 study participants per day on controlled diets. This capacity is sufficient to meet the targeted recruitment goals.

There is an additional ~250 sq ft laboratory space in Wallace Hall housing stadiometers/scales, tables for completing questionnaires, a private room for measuring body weight/height, and a file storage area. There is a research technician office with a desktop computer and printer, and two additional computer workstations with Internet access, NDS-R dietary analysis and SPSS statistical analysis software. All laboratory computers have external hard drives and cloud-based backup systems for automated daily data backups. The building has a conference room down the hall from the Metabolic Kitchen, and a participant waiting area and patient counseling area one floor below the Metabolic Kitchen. Wallace Hall is located on a public bus route, and three large parking areas are available around the building for private vehicle parking. The study will have parking spaces dedicated to this project adjacent to Wallace Hall, at no charge to participants.

Fralin Biomedical Research Institute

Dr. DiFeliceantonio’s lab is part of the Fralin Biomedical Research Institute at Virginia Tech Carilion (FBRI at VTC), located in Roanoke, Virginia. The Research Institute was

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

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

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

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

Computing Resources:

The Fralin Biomedical Research Institute (FBRI) at Virginia Tech Carilion (VTC) houses the following shared computing resources available:
Dell / Intel HPC Cluster (20 nodes, 960 Core, 3,840 GB RAM, 40Gb Interconnect) – SLURM; IBM iDataPlexLinux Cluster (60 node, 720 core Intel Xeon based, 1,440GB RAM, GigE Interconnect) - PBS; 10+ dedicated Linux servers (48 core AMD Opteron-based, 192GB RAM) are available for image and data analysis; Virtualized infrastructure using VMware vSphere, virtual machine environment available for general compute and image and data analysis; 40Gbit/s storage connectivity for research data; 10 Gbit/s

Internal Local Area Network between file servers and cluster; 8Gbit/s Fibre Channel Storage Area Network; 10 Gbit/s Wide Area Connection for access to Virginia Tech main campus / Internet; 1.4 Petabytes of NAS centralized disk storage; 250 Terabytes of SAN-attached centralized disk storage; 400 Terabyte library-based tape backup; Nightly backups and snapshots; 2.4Ghz / 5Ghz secure wireless network; 1 Gbit/s commodity Ethernet network; Data Analysis Tools: MATLAB, SPM12, AFNI, FSL, MRIcro, xjView, R, SAS, Prism Graphpad, SPSS; Productivity Tools: Adobe Suite, Microsoft Office Suite, vi, vim, emacs

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

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.

N/A