Cover page for Clinicaltrials.gov

Research Protocol
Official title: Short Term Choline Supplementation and Cardiovascular Health in Adults
NCT#: NCT04764162
Document date: 8/10/2021

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.

Short Term Choline Supplementation and Cardiovascular Health in Adults

PROTOCOL NUMBER:

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

18-535

PRINCIPAL INVESTIGATOR:

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FUNDING:

Sponsor(s): National Institute of Health
Funded already or in the proposal phase?: Awarded and new Award in progress
Is Virginia Tech the primary awardee or the coordinating center of this grant or contract? If not, list the primary institution: Click here to provide a response.

VERSION NUMBER/DATE:

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

1.0: 10/20/18 WIRB

2.0: 1/30/2020

3.0: 4/16/2020

4.0: 8/10/2021

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?
1.0	10/20/18	Initial submission to WIRB transfer to BRANY	N
2.0	1/30/2020	Change of Study Site Location to: 1872 Pratt Dr, Suite 1575 Garvin Bldg, Blacksburg, VA 24060	Y
3.0	4/16/2020	New funding source, significant changes to protocol new measurements.	Y
4.0	8/10/2021	Changes to protocol, flyer and informed consent form	Y

Table of Contents

1.0 Study Summary

Study Title	Short Term Choline Supplementation and Cardiovascular Health in Adults
Study Design	2 x 4 crossover design with repeated measures and we will conduct descriptive univariate analyses on all study variables.
Primary Objective	To establish proof-of-concept for a greater reduction in glucose homeostasis following increases in gut microbiota-generated TMAO on glucose homeostasis in older compared with young adults in order to conduct a larger, more comprehensive and mechanistic trial in the future.
Secondary Objective(s)	- Obtain preliminary data for effect size generation.
Study Population	We are recruiting men and women who are between the ages of 18-79 years of age. We will recruit individuals of all races and ethnic backgrounds.
Sample Size	60 subjects
Research Intervention(s)/ Investigational Agent(s)	-Interventions (Choline and Placebo supplementation in capsule form)
Study Duration for Individual Participants	*How long will a participant spend in your study? If multiple sessions, list time required for each session plus total.* Visit: 1 = 1 hour Visit: 2, 8, 11, 17 = 3 hours each Visit: 3, 9, 12, 18 = 2 hours each Visit: 4, 10, 13, 19 = 3 hours each Visit: 5, 6, 7, 14, 15, 16 = 30 minutes each Total Time: 36 hours
Acronyms and Definitions	TMA = Trimethylamine TMAO = Trimethylamine-N-oxide T2D = Type 2 Diabetes GI = Gastrointestinal FMD = Flow mediated dilation CGM = Continuous glucose monitor

2.0 Objectives

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

To establish proof-of-concept for a greater reduction in glucose homeostasis following increases in gut microbiota-generated TMAO on glucose homeostasis in older compared with young adults in order to conduct a larger, more comprehensive and mechanistic trial in the future.

2.2 State the hypotheses to be tested:

We hypothesize that gut microbiota-generated increases in plasma TMAO will be greater in older compared with young adults and this will be associated with a correspondingly greater reduction of insulin sensitivity, mixed meal tolerance, and 24-hr glycemic control in older adults.

In addition, we hypothesize that the magnitude of change in gut microbiota-generated increases in TMAO and measures of insulin sensitivity, mixed meal tolerance, and 24-hr glycemic control with the intervention will be associated with the magnitude of change in the abundance of TMA-producing bacteria and/or expression of cutC TMA lyase.

3.0 Background

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

Dysbiosis of the gut microbiota, characterized as adverse changes in the abundance and/or composition of gut microbes, has been implicated in pathophysiology of a number of chronic diseases including neurological disorders, colitis, atherosclerosis, and metabolic diseases. Several lines of evidence in both animal models and humans also implicate gut dysbiosis in the etiology of insulin resistance and T2D. Future studies are needed to understand the potential link between gut microbial composition and function with glucose homeostasis and risk of T2D. Recently, gut microbiota-generated increases in TMAO has become a novel target for the treatment of cardiovascular diseases. The results of studies in rodent models indicate a causal role of TMAO in the development of atherosclerosis and several meta-analyses support a dose-dependency of TMAO in predicting adverse CVD events. Given the close relationship between T2D and cardiovascular disease, perhaps it is not surprising that a higher prevalence of T2D has been observed in populations with elevated TMAO. The potential role of TMAO in the development of insulin resistance and T2D is supported by multiple observations. Changes in gut microbial composition with aging are observed even in the absence of overt disease. However, the role of these changes on the reduced glucose homeostasis observed with aging is unknown. Interestingly, TMAO increases with advancing age in both mice and humans. Human TMAO increases are believed to be a consequence of changes in the gut microbiota, i.e., dysbiosis. Indeed, there is an increase in the abundance of TMA-producing bacteria with aging. Importantly, and as emphasized earlier, plasma TMAO concentrations are dependent on the composition of the gut

microbiota and higher baseline TMAO concentrations are associated with larger increases in TMAO with choline loading. As such, we hypothesize that older adults will demonstrate greater gut microbiota generated increases in TMAO following choline loading compared with young adults. Furthermore, the greater gut microbiota-generated increases in TMAO following choline loading will be associated with not only a greater abundance of TMA-producing bacteria but a larger decrement in insulin sensitivity, mixed meal glucose tolerance, and a reduction in 24-hr glycemic control.

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:

The premise of the present proposal is that: 1) advancing age is associated with increases the abundance of TMA producing bacteria and TMAO concentrations; 2) older adults are at increased risk of insulin resistance, glucose intolerance, and T2D; and 3) there is accumulating evidence that elevated TMAO levels are predictive of, and contributory to, risk of T2D; and 4) the impact of gut microbiota-generated increases in TMAO on age-related changes in glucose homeostasis is unknown. Our proposal has significant translational potential as our findings will advance basic science findings in mouse models to determine relevance in humans and provide novel mechanistic insight into meta-organismal nutrient metabolism as a basis of age-related reductions in glucose homeostasis. One of the major challenges in understanding this link is the potential confounding influence of habitual dietary intake on key outcome variables. We will overcome this limitation by leveraging our existing expertise in rigorously standardizing participant diets to isolate the effects of meta-organismal metabolism of dietary choline as a basis for elevated risk of T2D with advancing age using state-of-the-art and reproducible methodology. Furthermore, our study will provide important insight into the gut microbiota as an important source of variability in glucose metabolism to TMAO. Our findings could have important policy and clinical implications. If modifying the gut microbiota to reduce age-related T2D risk is prudent, then clinical practice could be advanced by informing treatment guidelines and by providing rationale for individualizing nutritional or pharmaceutical interventions that target the gut microbiota as the interface between TMAO and T2D risk.

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

With this study, there are three continuous measurements which will define our study endpoints. These include oral glucose tolerance, flow mediated dilation of the brachial artery and aortic pulse wave velocity measurements corresponding to arterial stiffness and a mixed meal tolerance test.

With regards to our hypothesis stated in section 2.2, our study endpoints will be as follows: Gut microbiota-generated increases in plasma TMAO will be greater in older compared with young adults and this will be associated with a correspondingly greater reduction of insulin sensitivity, mixed meal tolerance, and 24-hr glycemic control in older adults.

*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.)*:

Our safety endpoints will correspond to the potential risks that subjects may experience which are stated in section 17.1.

Potential safety endpoints which could occur with this study are as follows: 1) Any severe GI distress or complaints reported with consumption of the choline or placebo supplement, 2) Discomfort or pain which is not tolerated by the subject with blood draws, 3) Discomfort and pain due to the inflation of the BP cuff on the forearm of the participant; and 4) Discomfort which is reported when measuring arterial stiffness measurements due to slight pressure being applied to the carotid, brachial, radial and femoral arteries.

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):

A double-blind randomized 2x3 crossover design with 3 repeated measures in each period will be used. Pre-/post-test evaluation used in each period to evaluate the ingestion of choline/placebo on the effects to cardiometabolic health.

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):

Data will be examined for the presence of outliers, violations of normality and missing data. Major violations of normality will be corrected with an appropriate transformation procedure. In case of an outlier, rather than transform the data, the outlier will be "Winsorized", that is, replaced by the most extreme value in the tail of the distribution. Data will be checked for sequence, period and carryover effects. Next, a mixed-effects linear model (using SAS PROCMIXED) will be used to estimate the treatment differences between choline and placebo (H0: $\mu AB - \mu BA = 0$). Because of the two repeated measurements of the dependent variable in each period, we will treat the multiple observations as nested within individuals. This will allow us to model the various sources of intra-person and inter-person variability, as well as account for the correlation in the nested data to make the correct inference regarding differences between treatments. Confidence intervals (95%) and bootstrapped standard error estimates will be reported for statistical precision and estimation of effect sizes for a larger more comprehensive trial. An identical approach will be used for analysis of other outcomes (e.g., arterial stiffness, etc.).

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.

- Subjects will be recruited from the New River Valley and surrounding areas.
- The study will be conducted in the Department of Human Nutrition, Foods, and Exercise at Virginia Tech, Blacksburg, VA. Participants will be required to come to Garvin Building, Suite 1575 (1872 Pratt Dr., Blacksburg, VA 24060) for 19 visits.

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.*

This study will utilize the use of choline supplement (500mg BID capsules) and a same look/taste placebo supplement (500mg BID capsules) randomized in a double blinded trial as interventions.

The choline and placebo supplements will be stored and locked away in the Human Integrative Physiology Lab and only members of the research team will have access to these capsules. Once subjects are required to be provided with the choline or placebo supplement, they will be provided to the participants in sealed containers which are assigned by subject ID. Only members of the research team will provide the supplements to the subject.

The capsules will be produced in aseptic conditions in the Human Integrative Physiology Laboratory by members of the research team.

DEXA scans will be used as part of a body composition analysis required on four visits (Visit 3, 9, 12, 18). The amount of radiation that a DEXA exam incurs is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount that will be received is equal to 4*1/20 of a chest x-ray.

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:

Choline: Choline is a water-soluble vitamin-like essential nutrient that is available as an over-the-counter supplement. Adequate intake for adults is 550 mg/day for men and 425 g/day for women. Tolerable upper intake level for adults is 3500 mg/day. Doses over the daily upper intake levels may cause side effects such as sweating, a fishy body odor, gastrointestinal distress, diarrhea, and vomiting. Some individuals might be allergic to choline or the other ingredients. Individuals who have known allergies to choline or other ingredients will be excluded. The subjects always will have the ability to terminate their participation for any reason without consequence. The dose of 1000 mg/day dose of choline bitartrate USP (500 mg BID; NutriScience, Trumbull, CT) provided is well below the tolerable upper intake level for adults. Choline bitartrate USP is available as an over-the-counter supplement and is Generally Recognized as Safe by the FDA.

Placebo: This will mimic the look of the choline capsule and contains 1000 mg (500 mg BID) of maltodextrin, a polysaccharide or carbohydrate commonly found in foods in a normal diet.

Nitroglycerin: These are stabilized sublingual compressed tablets which will contain 0.4mg of nitroglycerin and are FDA approved.

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:

- To perform the FMD portion of the investigation, duplex ultrasonography will used. To perform this, a GE Vivid S6 unit equipped with a high-resolution linear array transducer will be used in accordance with published guidelines. Furthermore, carotid imaging will be performed using the same unit to obtain carotid artery diameters.

- To perform the arterial stiffness measurements, applanation tonometry will be used. A fingertip probe which is equipped with a high-fidelity strain gauge transducer will be placed on the carotid, brachial, radial, and femoral arteries to obtain pressure waveforms and amplitudes. Again, this procedure will be performed following published guidelines.

- Continuous measurements of glucose will be assessed using the FreeStyleLibre Pro, Abbott CGM. It will be placed on the upper back side of an arm. This will be performed following the guidelines and protocol from the device's company (Abbott).

- Blood pressure measurements will be obtained using a mercury sphygmomanometer or automated blood pressure monitor and these measurements will strictly conform to American Heart Association guidelines.

- Body composition analysis will be obtained via a DEXA scan with a Prodigy Advance, GE Healthcare unit. This will be performed only by trained and certified technicians and the amount of radiation that a DEXA exam incurs is less than the amount permitted by the FDA per year.

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***	

IDE exempt

8.0 Procedures Involved

8.1 Describe and explain the study design:

Using a randomized cross-over design, participants will be given and asked to consume either choline or a placebo (maltodextrin) supplement for four weeks and will consume 1000 mg (500 BID) of choline or placebo per day. Testing will take place over twelve visits. Participants will complete a screening visit and two periods of three-day testing sessions before and after the four-week period of choline and placebo supplement. In between experimental sessions, there will be a two-week "washout".

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.*

Session 1: Baseline (screening) session (Approximately 2 hours) Garvin Building, Suite 1575

Overnight Fast: Subjects will be asked to avoid eating or drinking anything except water and to avoid consuming any caffeine-containing beverages for 12 hours prior to this visit so that the test results will not be influenced by the food they eat or by the normal digestion process.

Blood Chemistry: A blood draw will be collected for choline concentrations as well glucose, cholesterol, and other factors that may impact or be reflective of vascular function/health. A needle will be inserted in an arm vein to draw blood (approximately 2 tablespoons/draw).

Urine Test: Subjects will be asked to urinate in a small cup. Sodium and other electrolytes, glucose, protein, pH, and whether there are blood cells present will be measured to determine whether it is safe for each individual to participate in the study.

Proof of COVID-19 vaccination: Subjects will be asked to bring their vaccination card against COVID-19 to the laboratory.

Medical History: Subjects will be asked to complete a medical history questionnaire, which will be used to screen for health problems (unstable heart disease) or other reasons (medications which influence study results) that would preclude participation.

Physical Activity: Subjects will be asked to complete a questionnaire regarding usual daily activities. They will also be asked to wear a device called an accelerometer over a 4-day period to measure their physical activity level

Infection/Inflammation Questionnaire: Subjects will be asked to complete a questionnaire about any recent illnesses or infections that they may have had in the prior month.

Dietary Record: Subjects will be asked to record/recall all of the food they eat during a 4-day period.

Body mass and height: Body weight will be measured on a digital scale accurate to +0.01 kg. Height will be measured with a stadiometer

Resting Blood Pressure and Heart Rate: Blood pressure measurements will be made under quiet, comfortable ambient laboratory conditions via mercury sphygmomanometry or an automatic blood pressure monitor. Heart rate will also be determined by an automatic blood pressure cuff. Measurements will conform strictly to American Heart Association guidelines.

Session 2, 8, 11, 17: (Approximately 3 hours) Garvin Building, Suite 1575

Overnight Fast: Subjects will be asked to avoid eating or drinking anything except water and to avoid consuming any caffeine-containing beverages for 12 hours prior to this visit so that the test results will not be influenced by the food they eat or by the normal digestion process.

Resting Blood Pressure and Heart Rate: Blood pressure measurements will be made under quiet, comfortable ambient laboratory conditions via mercury sphygmomanometry or an automatic blood pressure monitor. Heart rate will also be determined by an automatic blood pressure cuff. Measurements will conform strictly to American Heart Association guidelines.

Body Mass: Body weight will be measured on a digital scale accurate to +/- 0.01 kg.

Dietary intake analysis (24-hour recall): Subjects will be asked to recall all the food and beverages they consumed in the previous 24-hour period.

Oral Glucose Tolerance Test: One small plastic tube (catheter) will be placed in one of the subject's arm veins. After a baseline blood draw to measure glucose, insulin, cholesterol, and other factors that may impact or be reflective of the subject's health status (e.g., C-reactive protein), the subject will drink a sugary drink of glucose (75 grams) and blood will be collected in small amounts (less than one half teaspoon) every half hour (4 blood draws total) for a 2-hour period.

Stool Collection: Subjects will be given a test tube and asked to collect a stool sample. Subjects will be asked to bring the sample back to the laboratory during their next scheduled visit. All supplies and instructions will be provided by the laboratory.

Continuous Glucose Monitoring: Glucose will be measured by a continuous glucose monitor (FreeStyle Libre Pro, Abbott). A small sensor will be placed on the subject's skin overlying the back side of their arm in order to measure glucose continuously. The sensor has a small plastic tube that is inserted into the tissue just below the skin and is held in place with sticky tape. The device will be worn for 3-4 days.

Session 3, 9, 12, 18: (Approximately 2 hours) Garvin Building, Suite 1575

Overnight Fast: Subjects will be asked to avoid eating or drinking anything except water and to avoid consuming any caffeine-containing beverages for 12 hours prior to this visit so that the test results will not be influenced by the food they eat or by the normal digestion process.

Resting Blood Pressure and Heart Rate: Blood pressure measurements will be made under quiet, comfortable ambient laboratory conditions via mercury sphygmomanometry or an automatic blood pressure monitor. Heart rate will also be determined by an automatic blood pressure cuff. Measurements will conform strictly to American Heart Association guidelines.

Body Mass: Body weight will be measured on a digital scale accurate to +/- 0.01 kg.

Dietary intake analysis (24-hour recall): Subjects will be asked to recall all the food and beverages they consumed in the previous 24-hour period.

Blood Chemistry: A blood draw will be collected for choline concentrations as well glucose, cholesterol, and other factors that may impact or be reflective of vascular function/health. A needle will be inserted in an arm vein to draw blood (approximately 2 tablespoons/draw).

Arterial Stiffness: Carotid Ultrasonography - Common carotid artery diameters will be measured from the image obtained from an ultrasound unit (GE Vivid S6) equipped with a high-resolution linear array transducer. Applanation Tonometry - The carotid, brachial, radial and femoral artery pressure waveform and amplitude will be obtained by a fingertip probe incorporating a high-fidelity strain gauge transducer.

Brachial Artery Flow Mediated Dilation (FMD): FMD of the brachial artery will be assessed using duplex ultrasonography (GE Vivid S6) with a high-resolution linear array transducer according to published guidelines. Reactive hyperemia will be produced by inflation of a pediatric BP cuff around the forearm for 5 minutes. Offline analysis of baseline and post-reactive hyperemic diameters and velocities will be performed using edge detection software (Vascular Analysis Tools, Medical Imaging Applications, Inc.).

Endothelium Independent Vasodilation: This will be assessed by measuring brachial arterial dilation for 5 minutes following administration of 0.4 mg of sublingual nitroglycerine. Individuals will not be allowed to participate in this aspect if they take Cialis or Viagra or have a history of coronary heart disease.

24-hour Urine Collection: Subjects will be asked to collect all urine samples over a 24-hour period and bring the sample back to the laboratory during their next scheduled visit. All supplies and instructions will be provided by the laboratory.

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 dual-energy x-ray absorptiometry (DEXA) (Prodigy Advance, GE Healthcare).

Pregnancy Test: If a subject is female and pre- or peri-menopausal or not postmenopausal for at least 1 year, she will be required to provide a small cup of urine for a pregnancy test.

Session 4, 10, 13, 19: (Approximately 3 hours) Garvin Building, Suite 1575

Overnight Fast: Subjects will be asked to avoid eating or drinking anything except water and to avoid consuming any caffeine-containing beverages for 12 hours prior to this visit so that the test results will not be influenced by the food they eat or by the normal digestion process.

Resting Blood Pressure and Heart Rate: Blood pressure measurements will be made under quiet, comfortable ambient laboratory conditions via mercury sphygmomanometry or an automatic blood pressure monitor. Heart rate will also be determined by an automatic blood pressure cuff. Measurements will conform strictly to American Heart Association guidelines.

Body Mass: Body weight will be measured on a digital scale accurate to +/- 0.01 kg.

Dietary intake analysis (24-hour recall): Subjects will be asked to recall all the food and beverages they consumed in the previous 24-hour period.

Mixed Meal Tolerance Test: Asmall plastic tube (catheter) will be placed in one of the subject's arm veins. After a baseline blood draw to measure glucose, insulin, cholesterol, and other factors that may impact or be reflective of the subject's health status (e.g., C-reactive protein), the subject will drink a mixed meal beverage (Ensure original plus ®, 350 calories, 48g carbohydrate, 11g fat, and 16g protein) and blood will be collected in small amounts (less than one half teaspoon) at timepoints 0, 30, 60, 90, and 120 over a two-hour period.

Continuous Glucose Monitoring: The device which was placed on the subject will be removed.

Choline Supplement/Placebo: Subjects will be given enough capsules to last one week and asked to consume 1000 mg (2 x 500 mg tablets) of choline or placebo with a bottle of water every day.

Sessions 5-7, 14-16: (Approximately 30 minutes per session) Garvin Bldg, Suite 1575

Choline Supplement/Placebo: Subjects will be given enough capsules to last one week and asked to consume 1000 mg (2 x 500 mg tablets) of choline or the placebo with a bottle of water every day.

Resting Blood Pressure and Heart Rate: Blood pressure measurements will be made under quiet, comfortable ambient laboratory conditions via mercury sphygmomanometry or an automatic blood pressure monitor. Heart rate will also be determined by an automatic blood pressure cuff. Measurements will conform strictly to American Heart Association guidelines.

Body Mass: Body weight will be measured on a digital scale accurate to +/- 0.01 kg.

Dietary intake analysis (24-hour recall): Subjects will be asked to recall all the food and beverages they consumed in the previous 24-hour period.

After session 10: Following this session, subjects will return to their normal activities for a two-week "washout".

8.3 Describe:

- *Procedures or safeguards intended to reduce the probability and magnitude of risks. (For example: Reducing the risk of injury in a virtual reality study either by having the subjects sit during the study or by providing an obstacle-free space for walking.)*
- *Be sure to describe all drugs and devices used in the research, when they will be administered or used, and their purpose.*
- *Methods used to collect data about subjects. Please upload all data collection forms to Protocol Management. Some common examples are:*
  - *Screening questionnaires*
  - *Survey(s), including online surveys*
  - *Demographic questionnaire(s)*
  - *Interview guide(s), e.g., questions or pool of questions for semi-structured interviews*
  - *Focus group guide(s)*
  - *Other documents used to collect data*

- Blood Draw: The P.I. or a trained technician will perform all blood draws. Aseptic conditions will be followed during all of the procedures. Universal precautions will be taken in collection and handling of all blood samples. Subjects will be informed that their blood will be analyzed for presence of HIV and Hepatitis, if an experimenter is exposed to their blood.

- Endothelial Function: The risk of low blood pressure during nitroglycerine administration is minimized because the subject is already in the supine position. There is a small risk of a headache, but this will last only a few minutes. Individuals taking Cialis or Viagra cannot participate in this aspect of the study.

- DEXA scan: DEXA procedure will be performed by trained staff. Subjects will be informed of the risk of radiation exposure prior to study enrollment.

- Continuous Glucose Monitor: The placement of the CGM on the subject will be performed by trained staff. This will take place in aseptic conditions, minimizing the risk of any infection.

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:

- Study data will be collected on data sheets and manually entered into a database (Excel format) on a secure computer.

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)*

N/A

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:

All data will be stored in a locked cabinet in Dr. Kevin Davy's laboratory the Human Integrative Physiology Laboratory located in Garvin Building, Suite 1575 (1872 Pratt Dr., Blacksburg, VA 24060) which is also locked with access to authorized personnel only. The computer data will be stored in the locked lab on a computer that is password protected. All de-identified data will be kept indefinitely and there is no need to use specimens for the scope of the study.

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:

Codes using a combination of letters and/or numbers will be used to de-identify subjects from their personal information. This de-identified data collection sheet will be stored and locked away and the key will be kept in a locked cabinet separate from the subjects' completed data documents and accessibility will be limited to the PI and Co-PI's.
The people who will have access to this data are the investigators and students involved in the study
De-identified data may be kept indefinitely. Identifiable data and blood samples will be retained before being destroyed for 5 years.

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:

At the conclusion of the study or when the subjects' involvement in the study ends, they will be provided with their blood pressure readings, body composition results, and information on their dietary intake. This information will be provided in a summary of the study's findings.

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 duration of an individual's participation in this study will be approximately 12 weeks, which will include 19 visits to the HNFE department at Virginia Tech and approximately 36 hours of their time. The actual time and frequency of the subject's visits will depend on their and the study staff's schedule.
Subjects will begin the study on a rolling basis, but this will take place across approximately 12 months.
The total amount of time expected to complete this study is approximately 24 months.

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:

Those who respond to the investigation's advertisements will be asked to complete a brief online screening questionnaire to confirm basic eligibility criteria on Qualtrics (e.g., age, health status).
Furthermore, subjects will be screened for any possible health problems that would make it unsafe to participate, they will be required to complete a medical history questionnaire and made fully aware of their right to withdraw from the study at any time.

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 is as follows:
- 18-79 years of age
- Sedentary (low physical activity)
- Not diabetic
- Not taking medications that influence the study results
- Not have severe obesity (BMI <35kg/m^2)
- Non-smokers
- Not pregnant
- Without any major medical problems

- Fully vaccinated against COVID-19. Individuals are fully vaccinated 2 weeks after their final dose of an approved vaccine.

Exclusion criteria would be if individualss do not fall under any of the above criteria.

Therefore, subjects will be excluded if they are not between 18-79 years of age, pregnant, a smoker, have a BMI >35 kg/m^2. Additionally, if they have changed their dietary patterns in the last month, have unstable heart disease or diabetes, vegetarians, vegans, untreated high cholesterol, or have health problems that make it unsafe to participate

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*

All of the above will be excluded from the study.

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.*

This research study has the potential to include employees of Virginia Tech. However, during the consenting process of the research study, the participants will be made aware that only members of the research study will have access to their data and that this data will utilize a coding system making their data unidentifiable. This data will be locked away and they will be made fully aware of their right to withdraw from the study at any time.

14.0 Number of Subjects

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

We have powered our pilot study to detect a change in insulin sensitivity of 20 ±32% (effect size=0.63). We believe changes in other outcomes will be of similar magnitude. We will enroll 60 participants. Importantly, the purpose of this pilot study is to assess feasibility, acceptability, and provide proof of concept. Even with a small sample size (n=60), a cross-

over design with 4 repeated measures will give us fairly good estimates of the effect sizes, which is needed for a more comprehensive future trial.

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:

300-400 potential subjects will be screened for eligibility in this study. Of this 300-400 individuals, 60 participants who are eligible will be enrolled to take part in the investigation.

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

All subjects will complete each procedure.

15.0 Recruitment Methods

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

Recruitment will take place immediately following IRB approval. We are looking to recruit subjects from the New River Valley and surrounding areas. We will recruit subjects via posted fliers, newspaper advertisements, web postings, and community blood pressure screenings. Additionally, we plan to contact previous study participants/people who have expressed interest in future studies and who gave us permission to contact them for this purpose.

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):

We want to recruit from the general population.

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

Posted fliers, newspaper advertisements, web postings, and community blood pressure screenings. Additionally, we plan to contact previous study participants/people who have expressed interest in previous studies who gave us permission to contact them for future studies. Potential subjects will also be filtered via the use of Qualtrics as a basic screening for eligibility and potential subjects to follow up on.

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.*

Subjects may receive up to $400 in compensation.

Subjects will receive $20 for completing each testing session 2-18. After completing session 19, subjects will receive $60. There will be no compensation for completing the first screening session

16.0 Withdrawal of Subjects

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

Circumstances which we anticipate that could result in withdrawal are if a subject fails to follow instructions or adhere to the investigations intervention, and if they are late or miss scheduled appointments.

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

If a participant is terminated or discontinued from the study for reasons as described above, the PI will mitigate issues leading to these problems. Additionally, the PI will discuss these difficulties with the participant and explain the importance of adhering to the intervention for the purpose of the study. The participant will be provided with the compensation that is due and provided their information which is available to them (blood pressure, body composition and dietary intake). It will then be suggested that the study personnel part ways with the participant.

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):

If a subject who is enrolled into the investigation is taking Cialis and/or Viagra, they will not be allowed to participate in the endothelial function aspect of the research which involves the sublingual use of nitroglycerin. However, they will be allowed to participate in all other aspects and the use of such medications will not be a determining factor in the inclusion/exclusion of the subject.

In the rare chance in which there could be difficulty with the placement of the catheter in order to obtain blood draws for the oral glucose tolerance test, blood samples may then be performed via finger stick to collect glucose level measurements.

If the subject is uncomfortable with any aspect of having blood drawn, they have the opportunity to voluntarily pull out of any/all of the blood draws and will be allowed to continue with other aspects of the study.

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)*

Choline: Choline is a water-soluble vitamin-like essential nutrient that is available as an over the counter supplement. Adequate intake for adults is 550 mg/day for men and 425 g/day for women. Tolerable upper intake level for adults is 3500 mg/day. Doses over the daily upper intake levels may cause side effects such as sweating, a fishy body odor, gastrointestinal distress, diarrhea, and vomiting. Some individuals might be allergic to choline or the other ingredients. Individuals who have known allergies to choline or other ingredients will be excluded. The subject always will have the ability to terminate their participation for any reason without consequence. The dose of 1000 mg provided is well below the tolerable upper intake level for adults.

Blood Draw: Some pain or discomfort may be experienced when the catheter is inserted in the vein, but this should persist for only a short time. During the blood draws, subjects may have pain and/or bruising at the place on their arm where the blood is taken. In about 1 in 10 or 10% of the cases, a small amount of bleeding under the skin will cause bruising. The risk of a blood clot forming in the vein is about 1 in 200, while the risk of infection or significant blood loss is 1 in 1000. There is a small risk of the vein becoming inflamed and/or painful in the hours or days after the needle is removed. If subjects feel faint during or after a blood draw, they will be informed to notify the study doctor or study staff immediately and lie down right away to avoid falling. Having staff who are experienced in venipuncture and blood draws will minimize these risks.

Oral Glucose Tolerance Tests: This procedure requires the placement of a catheter in an arm vein, the risks here are identical to that stated above. In addition, there is a small risk of low blood sugar occurring during or after the test. We will be monitoring subjects blood sugar frequently and can usually anticipate before blood sugar drops too low. If this happens, orange juice (with table sugar) or some other simple carbohydrate containing food will be provided. We will monitor the individual's glucose until it returns to normal.

DEXA Scan: The amount of radiation that subjects will receive in the DEXA exam is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount subjects will receive is equal to 1/20 of a chest x-ray. The more radiation an individual receives over the course of their lifetime, the more likely that individual's risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks, however the exact increase in such risk is not known.

HIV/AIDS: In the event a researcher or other staff person is improperly exposed to the subject's blood, his or her blood will be tested for the presence of HIV, the Hepatitis B Virus, and the Hepatitis C Virus. There will not be any cost to the subject for this test. The research team will follow proper procedures for testing and reporting as outlined by Virginia State Law, which includes sending the individual to a doctor's office for a blood draw. Should the blood be tested positive, the affected individual will be informed of the results and provided with the opportunity to receive appropriate and timely counseling. In addition, the results, if positive, will be sent to the local health department.

Endothelial Function: Some pain or discomfort may be experienced when the blood pressure cuff is inflated and the participant may have pain and/or bruising at the place on the arm where the cuff was inflated but this is unlikely. However, these reactions are temporary and resolve within a short time after completing or stopping the procedure. There is a small risk that the subject will become lightheaded, dizzy, or faint following nitroglycerin administration. If these events occur, the feet of the subject will be elevated to alleviate the symptoms. Since they are already lying down, there is no risk of falling. These risks are minimized by having a trained individual perform the procedure.

Arterial Stiffness: There is a risk of slight discomfort due to very slight pressure being applied to the carotid artery during the ultrasound procedure and to the carotid, brachial, radial, and femoral arteries during the tonometry procedure.

Continuous Glucose Monitor: The placement procedure requires a sensor being inserted into the back side of the upper arm of the subject. When the wire-like sensor is deployed by the researcher, the subject can expect a sensation similar to a needle insertion for a blood draw. Potential risks associated here include discomfort during the insertion, pain, inflammation, redness, swelling, minor bleeding and minor infection at the site. These risks will be minimized by having a trained individual perform the procedure and this will be performed in aseptic conditions. Subjects might also experience these symptoms as a result of contact between the adhesive pad of the sensor and the skin. In rare cases, an infection can spread to other parts of the body. Allergic reactions can develop in response the adhesive used to keep the CGM in place. If these symptoms occur, subjects

have the ability to remove the CGM at-will and can anticipate that their symptoms will clear up within a short time.

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.)

Blood Draw: The P.I. or a trained technician will perform all blood draws. Aseptic conditions will be followed during all of the procedures. Universal precautions will be taken in collection and handling of all blood samples. Subjects will be informed that their blood will be analyzed for presence of HIV and Hepatitis if an experimenter is exposed to their blood.

Endothelial Function: The risk of low blood pressure during nitroglycerine administration is minimized because the subject is already in the supine position. There is a small risk of a headache, but this will last only a few minutes. Individuals taking Cialis or Viagra cannot participate in this aspect of the study.

DEXA scan: The DEXA procedure will be performed by trained staff. Subjects will be informed of the risk of radiation exposure prior to study enrollment.

Whilst in the laboratory, each subject will be constantly monitored by the investigators and asked at regular intervals about how they are feeling. Furthermore, they will be made aware of their right to withdraw or discontinue any aspect of the protocol at any time.

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:

N/A

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:

Subjects will receive information about their dietary intake, body composition and blood pressure.

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

There are no direct benefits of participation. Subjects will be informed that this is not a wellness or medical exam and to discuss any concerns about their health information with their personal physician.

19.0 Data Management and Confidentiality

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

To ensure quality control and validity of the data collected, all aspects of the research procedures will be conducted by trained technicians and all the procedures include reproducible techniques that are used on a regular basis by the PI's laboratory.

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 always be secured and deidentified. Only authorized and trained personnel will have access to study data. All data on electronic devices will be password protected in a locked office in a locked building. Study identifiers will be kept in a separate locked cabinet then data files.

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?*

Only unidentified data or specimens will be stored, they may be stored indefinitely in the Garvin Building, Suite 1575. Only members of the research team in the protocol will have access and the PI is responsible for receipt and transmission of the data.

There is no intention to share or transport data at this current time.

Personal identifiers will be destroyed after completion of all statistical analysis.

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):

The minimal amount of private information will be collected and once the necessary data is collected all data will be unidentifiable through the use of codes and remain confidential in a locked cabinet.

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):

For procedures requiring the placement of sensors on a sensitive area of the body, such as tonometry measurements (arterial stiffness) on the inner thigh, this will be performed by a same gender investigator.

There is a private changing area in our Human Integrative Physiology Laboratory for when individuals need to change into hospital gowns for the arterial stiffness and FMD measurements.

There is a private bathroom for subjects to use and for when they are required to provide a urine sample for urinalysis.

Sensitivity will be ensured when discussing pregnancy testing with subjects.

During the consenting process and throughout the investigation, subjects will be made fully aware of their rights to withdraw from a procedure or the study.

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:

No existing sources of information will be accessed.

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

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

We do not anticipate any required reporting from the conduct of our study. However, we will report any of the above if there is circumstantial or disclosed information to the above points.

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

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

21.1 Describe:

- *The plan to periodically evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe (e.g., periodic reporting to the IRB, establishing a data monitoring committee, reporting data monitoring committee findings to the IRB and the sponsor).*
- *What data you will review, including safety data, unexpected events, and data that show the ability to produce the intended results.*
- *How the safety information will be collected (e.g., with case report forms, at study visits, by telephone calls with subjects).*
- *The frequency of data collection, including when safety data collection starts.*

- *Who will review the safety data and with what frequency.*
- *The statistical tests for analyzing the safety data to determine whether harm is occurring.*
- *Any conditions that will trigger an immediate suspension of the research (e.g., a serious adverse event).*

The data safety monitoring plan (DSMP) for this study focuses on close monitoring by the principal investigator (PI) in conjunction with a safety officer (Jose Rivero, MD), along with prompt reporting of excessive adverse events and any serious adverse events (AEs) to the NIH and to the Institutional Review Board. All serious AEs will be reported by the PI within 48 hours of occurrence to the IRB.

Safety reports will be sent to the PI and to the safety officer. The Project Coordinator (Elaina Marinik, Ph.D.) 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. Subjects will be queried at the laboratory visits when supplements are provided as to any side effects or changes in health status that they experience. Any reported side effects will be discussed immediately with the PI and if necessary (i.e., serious adverse event), with Dr. Rivero. Out-of-range laboratory data (i.e., BP, bloodwork) will be discussed immediately with the PI and Dr. Rivero to determine if medical intervention or other actions are warranted. Subjects will be referred to their primary care physician 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 test meals, 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:

If a subject is injured as a result of this study, they will be informed to seek medical care. Neither the research team nor the University has money set aside to pay for any medical treatments that would be necessary if they are injured as a result of their participation in this study. Any expenses that they incur which includes emergencies and long-term expenses would be their own responsibility. They will be made aware that this limitation should be considered prior to beginning their participation in this study.

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:

There will be minimum transportation to and from the study location, but 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*

Those who respond to the study's advertisements will be asked to complete a brief online screening to confirm basic eligibility criteria on uQaltrics (e.g., age, health status). Those still interested and eligible to participate will be provided a consent document to review and consider. Those who continue to express interest in participating will be invited to an initial session to hear the details of study and potential risks associated with their involvement. They will be given a chance to ask any questions and have them answered to their satisfaction. Those who would like to participate will be required to sign the consent document.
A member of the investigative team will obtain consent from subjects. Dr. Kevin Davy will be responsible for oversight of all aspects of the study
The consent process will take place in the Human Integrative Physiology Laboratory in the Garvin Bldg and this will occur in the initial contact with the subjects, where the study will be explained to them and they will be emailed or receive a copy of the informed consent.

Subjects will be allowed to take a copy of the informed consent home with them to review or a copy will be mailed, emailed or faxed to them. They will return at a later date with the consent to ensure they have had enough time to review the consent and have any questions answered.

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.*

Non-English-speaking individuals are not being recruited. English is the only language spoken by the study personnel (all native and fluent speaking).

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).*

N/A

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:

Subjects will be asked to sign the consent form to make sure they understand what is being asked from them and the potential risks and benefits involved. A member of the study team will acknowledge the witnessing of the signature with signing the consent also.

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).*

- The PI commits 70% of his effort to research activities so is broadly available and in the laboratory on a daily basis.
- We have powered our pilot study to detect a change in FMD 1.0±1.5%. The purpose of the pilot study is to assess feasibility, acceptability, and provide proof of concept. Even with a small sample size (n=60), a crossover design with repeated measures will give us fairly good estimates of the effect sizes, which is needed for a more comprehensive future trial.
- The PI has a 20+ track year record (15 years at Virginia Tech) recruiting and studying participants for studies similar to these. We typically need to screen 10 individuals for every 1 participant enrolled.
- The PI oversees a laboratory with a clinical coordinator and 5-6 graduate students. He is in the laboratory on a daily-basis working with students and is involved in data collection.
- The Human Integrative Physiology Laboratory (Director: K. Davy) is located in the Garvin Bldg affiliated the Virginia Tech campus in close proximity to the Laboratory for Eating Behaviors and Weight Management in Wallace Hall. There is ~1000sq ft of laboratory space dedicated to the conduct of the proposed studies. The clinical physiology laboratory (~250sq ft), the physiology laboratory (~250sq ft), body composition laboratory (~150sq ft), echocardiography laboratory (~150sq ft), and biochemistry laboratory (~250sq ft) will be used specifically for the conduct of the studies associated with the present proposal.
- The laboratory staff are all BLS certified (including operation of an AED). The Virginia Tech Rescue squad is available (911) for on campus emergencies. Dr. Jose Rivero is the medical director of the laboratory and provides general oversight.
- All staff are trained on study procedures they are assigned to perform before any subject is enrolled. The PI and clinical coordinator provide oversight and approve which staff have received adequate training. The laboratory staff meet weekly with the PI and clinical coordinator to discuss the ongoing protocol and research procedures and review each staff member's duties and responsibilities. Retraining/refresher training is performed periodically to avoid drift.

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