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.

Exploring the Role of Peanuts in Enhancing Healthy Weight Gain in Athletic Individuals

PROTOCOL NUMBER:

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

21-561

PRINCIPAL INVESTIGATOR:

Full Name and Degrees: Dawnine Enette Larson-Meyer
Department: Human Nutrition, Foods, and Exercise
Telephone Number: 540-231-1025
Email Address: enette@vt.edu

FUNDING:

Sponsor(s): Peanut Institute
Funded already or in the proposal phase?: Recently Funded
Is Virginia Tech the primary awardee or the coordinating center of this grant or contract? If not, list the primary institution: Virginia Tech

VERSION NUMBER/DATE:

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

Version 6.0

REVISION HISTORY:

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

Revision #	Version Date	Brief Summary of Changes (i.e., the different sections)	Consent Change?
1	28 June, 2021	Requested additional or clarifying details added to sections 8 (most), 9.4, 10.0, 12.2 and 12.3, 15.4, 21.0, 24.1 and 25.2	Yes
2	25 July, 2021	Requested additional details added to sections 7.3, 8.3, 9 and 15.4. Typos corrected. DOB and a few edits made to consent, advertisement flyer and several study forms.	Yes
3	28 Sept, 2021	Completed changes suggested by Board specifically related to the addition of coronary artery disease as an exclusion factor (section 12) and clarification on the health history questionnaire (section 8.1 and 20), GPS (section 8.1) and pregnancy test with the DXA (Table in section 8 and in consent. Clarified payment info (discussed on IRB Call in section 15.4; Made requested changes to consent (which included carefully reading over for clarity and typos and editing factors related to the above); Made required changes to recruitment flyer; Added the doctoral student (previously listed as TBA) who will be in charge of the protocol (A Sanchez).	Yes
4	Nov 1, 2021	We have made minor editorial and logistic questions to the protocol and consent that includes: a) minor changes in screening (we will use HIPA compliant zoom vs telephone when possible) and the screening form (updated form uploaded); b) minor changes to the snack items offered (complete list uploaded), walking economy speed, VO2max protocol, and resistance training exercises; and c) inserted language that participants would not	Yes

		be informed of the macronutrient composition of the two snack groups. We also include new/updated participant instructions for use of the smartwatch, CGM and 24 h urine collection.	
5	25 January	We added information about the Eating Attitudes Questionnaire that will be used to help screen for eating disorders and disordered eating to sections 12.2 and 19.5. We uploaded the referral sheet that will be provided to anyone who scores higher than a 20 on this test.	Yes
6	7 April	We added abnormal serum lipids as an exclusion criterion (section 12.0, page 26). We added an additional questionnaire at baseline to evaluate vitamin D status as a baseline health outcome and a study exit survey at study conclusion (Table 1 and section 8.2). We clarified that we will also measure iodine concentration in the urine samples to help interpret thyroid and other hormone concentrations at baseline and study end. We would also like to increase our potential sample size to 40 from 32 to account for a possibility of drop outs and ensure we have a complete sample of 32 participants; we still plan on recruiting as close to half men and half women as possible with a balance between our two intervention groups. We also added an exit survey. We uploaded the Vitamin D Food Frequency and Lifestyle Questionnaire and the Exit Survey.	Yes

Table of Contents

1.0 Study Summary

Study Title	Exploring the Role of Peanuts in Enhancing Healthy Weight Gain in Athletic Individuals
Study Design	Randomly Assigned Intervention Study
Primary Objective	Determine the efficacy of the standard hypercaloric sports performance diet (500 kcal surplus energy, adequate protein and carbohydrate, judicious fat) and weight training regimen with excess calories from peanut foods or high-carbohydrate snack foods on weight and lean mass gains over 10 weeks in athletic men and women desiring weight gain.
Secondary Objective(s)	Secondary Aim: Evaluate the ease and acceptability of whole peanuts, peanut butter and peanut-based healthy snacks relative to high-carbohydrate snacks as part of the standard hypercaloric regimen. Exploratory Aim: Examine the effect of potential adaptive factors (increased resting metabolism, decreased appetite) and individual characteristics (sex, age, training history, somatotype) on weight and lean mass gains.
Study Population	Athletic and military men and women who desire weight gain primarily as lean mass to improve performance and/or effectiveness in sport/military endeavors.
Sample Size	32 (16 men and 16 women)
Research Intervention(s)/ Investigational Agent(s)	Men and women will be randomly assigned to a peanut intervention or control group (8 men and 8 women per group); Each group will undergo a 10-wk diet and weight training regimen designed to promote healthy weight gain.
Study Duration for Individual Participants	12 to 13 weeks including screening, baseline testing, the 10-week intervention and post-testing
Acronyms and Definitions	DXA, Dual-Energy X-ray Absorptiometry; TSH, thyroid-stimulating hormone; CBC, complete blood count; RMR, resting metabolic rate; REE, resting energy expenditure; VO2 max, maximal oxygen uptake during exercise, also known as aerobic capacity; Reps; repetitions; CGM, continuous glucose monitoring; ISAK, International Standards for Anthropometric Assessment; ACSM, American College of Sports Medicine; IUSCA, International Universities Strength and Conditioning Association; HIP Laboratory, Human Integrated Physiology Laboratory; NEM Laboratory, Nutrition and Exercise Metabolism Laboratory

2.0 Objectives

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

1) Determine the efficacy of the standard hypercaloric sports performance diet (500 kcal surplus energy, adequate protein and carbohydrate, judicious fat) and resistance training regimen with excess calories from peanut foods or high-carbohydrate snack foods on weight and lean mass gains over a 10-wk period in athletic men and women desiring weight gain.
2) Evaluate the ease and acceptability of whole peanuts, peanut butter and peanut-based healthy snacks relative to high-carbohydrate snacks as part of the standard hypercaloric regimen.
3) Explore the effect of potential adaptive factors (increased resting metabolism, decreased appetite) and individual characteristics (sex, age, training history, somatotype) on weight and lean mass gains.

2.2 State the hypotheses to be tested:

The standard hypercaloric sports performance diet (500 kcal surplus energy, adequate protein and carbohydrate, judicious fat) and resistance training regimen with excess calories from peanut foods or high-carbohydrate snack foods will result in an average weight gain of 1/2 pound/week in both men and women, with the majority of that gain as lean (fat-free mass) mass.

Weight gain will be more efficient when the excess calories are from peanut foods (compared to control, high-carbohydrate foods) which will be better accepted than the control snacks.

3.0 Background

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

Many athletes and military personnel desire weight gain primarily as lean mass to improve performance and effectiveness in sport/military endeavors. While much is known about the energy restriction required to reduce body fat, little is understood about energy and macro-nutrients needed to promote healthy gains in body weight and lean mass. Typically, athletes are encouraged to increase energy intake by 400-500 kcal/day with an emphasis on adequate protein (1.2- 2.0 g protein per kg body weight for athletes), adequate carbohydrate (Butterfield, Kleiner et al. 1995, Williams, Branch et al. 2017), and judicious inclusion of healthy fat-containing, energy dense foods (e.g., peanut butter, peanuts, nuts, avocado, and dried fruit) (Grandjean 1999, Williams, Branch et al. 2017). While it is well recognized that greater gains in body mass are possible in the first year of

intense resistance training, subsequent gains taper off. Lemon, for example, estimated that body mass may increase by as much as 20% in young male body builders and football players in the first year of training, with subsequent yearly gains of only 1 to 3% (Butterfield, Kleiner et al. 1995). In general, however, gaining 1/2 to 1 lb/wk is the recommended goal (Williams, Branch et al. 2017).

Previous Studies on Weight Gain. Despite its importance, healthy weight gain is an often-ignored area of sports and human performance nutrition. Surprisingly little research has been conducted to either help establish guidelines for healthy weight gain or validate the typical aforementioned text book guidelines. In fact, initial weight gain guidelines were established using estimates of the energy required to promote muscle tissue accretion (Williams, Branch et al. 2017) from studies conducted in malnourished (Spady, Payne et al. 1976) and experimentally overfed sedentary subjects (Forbes, Brown et al. 1986). Muscle tissue itself is only~700-800 kcal/lb due to its high water content (~75% water) with only ~20% of weight as protein (Slater, Dieter et al. 2019). The additional 500 kcal/day (Butterfield, Kleiner et al. 1995, Williams, Branch et al. 2017, Slater, Dieter et al. 2019) was established based on estimates of the National Research Council that ~5 kcal are needed to support accretion of 1 gram of tissue during growth (Williams, Branch et al. 2017), which corresponds to estimates from studies of malnourished and overfed participants (Spady, Payne et al. 1976, Forbes, Brown et al. 1986).

Although methods of weight gain in athletes were heavily discussed in the mid to late 90's (Butterfield, Kleiner et al. 1995, Grandjean 1999), few studies have been conducted over the past 20 years, other than those specifically focusing on protein intake and timing on acute muscle protein synthesis (Phillips and Van Loon 2011). In one of the few studies conducted, Bartels and colleagues (Bartels, Lamb et al. 1989) found that the addition of 500 extra kcal/day during 9 weeks of heavy resistance training resulted in approximately ½ lb increase in lean mass/wk with no corresponding increase in body fat percentage in 12 male weight trainers. Body weight increases did not differ whether the hypercaloric diet was 45 versus 65% carbohydrate. Another semi-controlled study of Kreider et al. (Kreider, Klesges et al. 1996) examined whether supplementing the diet with one of two commercially-available weight gain powders for 28 days compared to a maltodextrin placebo influenced lean tissue accretion. Carbohydrate ingestion alone promoted a non-significant increase in lean weight of 1.47 lb (670 g) with increase in body fat weight; while ingestion of one commercial powder produced identical increases (665 g) but also an increase in body fat. The other powder promoted a significant increase of 1.56 lb (707 g) predominately as lean tissue. A more recent study by Garthe et al (Garthe, Raastad et al. 2011) examined the effect of nutritional counselling (designed to create a 500 kcal/day surplus) compared to ad libitum dietary intake on changes in body composition during an 8-12 week focused resistance training period in 21 elite Norwegian athletes (including 4 women). Individually-prescribed hypercaloric meal plans and counseling resulted in successful short and long-term gains in total body and lean mass. To our knowledge, no studies have evaluated intentional weight gain in active women (who may also desire weight gain (Minnick, Raffoul et al. 2020)) or focused on the health implications of such gain in athletes.

Benefit of Peanuts and Peanut Products in Healthy Weight Gain. Research and observations by sports dietitians suggest that active individuals interested in weight gain

often rely on commercially-available powders (Kreider, Klesges et al. 1996), energy bars and other high-protein specialized products as a source of additional calories (Minnick, Raffoul et al. 2020) during weight gain attempts. These food items, however, are considered ultra-processed foods (Pagliai, Dinu et al. 2021). A growing body of evidence links ultra-processed food consumption to adverse health outcomes, including increased risk of cardio-metabolic disease, stroke and depression (Pagliai, Dinu et al. 2021). Given the increased interest in the health benefits of whole-food and plant-based diets (and the combination of the two), peanuts are an ideal source of high-quality plant-based protein for consumers interested in weight/lean mass gain. Peanut butter, peanuts, and nuts are typically suggested foods to include in healthy weight gaining meal plans (Grandjean 1999) because they are both nutrient- and energy-dense. Peanuts contain nutritionally significant quantities of potassium, magnesium, copper, iron, and fiber (Kholief 1987). Peanut protein provides substantial quantities of most amino acids, but it is low in methionine and tryptophan, and particularly high in arginine relative to other proteins. Additional methionine and tryptophan are easily obtained by combining peanuts with oats, rice, wheat and nuts. The high arginine content along with high concentrations of phytonutrients may offer health benefits and reduce risk of chronic and inflammatory diseases. Arginine in particular is a precursor to the potent vasodilator, nitric oxide. External nitric oxide has the potential to lower blood pressure and promote exercise efficiency (Jones, Vanhatalo et al. 2021). Additionally, peanuts and peanut butter are affordable, readily available, and add flavor and versatility to the diet, making peanuts an ideal food to incorporate into a healthy weight gain meal plan.

3.2 Describe any relevant preliminary data:

Dr. Davy has previously completed a similar diet-induced weight gain protocol in non-athletes; This protocol overfed 14 male (Gentile, Orr et al. 2007) and 3 female (unpublished data) participants ~1000 calories per day over baseline requirements using commercially-available food/beverages as the source of excess daily energy.

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:

With increased prevalence of overweight and obesity in the U.S and worldwide, emphasis on understanding healthy weight gain in both the general population and among athletes and military personnel has been largely ignored. However, a recent survey of close to 977,000 Canadian adults, aged 17-32 years, found that ~23% of men and 6% of women reported attempting to gain weight in the past 12 months (Minnick, Raffoul et al. 2020). In athletes (Garthe and Maughan 2018) and military personnel (Bukhari, DiChiara et al. 2021), desire to gain weight is a top reason reported for supplement use. Attempts to gain weight included eating more overall and eating more protein (Minnick, Raffoul et al. 2020) through consumption of foods that are often ultra-processed, and likely to

have negative health effects. Previous studies by Dr. Davy's group found that weight gain of 5 kg using such foods increased systolic and diastolic blood pressure even in those who were relatively fit (Gentile, Orr et al. 2007). The proposed study would serve as an important first step in helping understand the gaps in knowledge related to healthy weight gain. Findings would be of interest to sports and military dietitians and would be expected to lead to better understanding of the energy surplus needed to overcome metabolic adaptation and develop more targeted weight prescriptions. Results would inform the design of more effective weight gain meal plans, not only in athletes and military personnel, but also in older individuals and in clinical populations where promotion of weight gain is advocated. Future trials based on findings of the proposed study will likely explore benefits of peanut products in healthy weight in a variety of populations

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 Endpoints

- Change in Body Weight
- Change in Fat-Free Mass
- Change in Fat Mass (via DXA)
- Starting and Ending Body Fat Percentage (via DXA)
- Change in Sum of Skinfolds
- Change in Body Circumferences/Girth
- Absolute Energy Intake and Change in Energy Intake
- 24-h Glucose
- Snack Susceptibility and Ease of Consumption Rating

Secondary Endpoints

- Change in Resting Metabolic Rate/Resting Energy Expenditure
- Change in Hormone and Metabolic Markers in Blood including testosterone (free & total), growth hormone, insulin-like growth factor, leptin, insulin, thyroid hormones, cortisol and ghrelin and urinary creatinine and urinary iodine
- Change in Strength (all muscle groups)
- Change in Aerobic Fitness
- Change in Walking Economy

Change in and absolute values of Health Outcomes (blood pressure, serum cholesterol, fasting and 24-hour glucose concentration, and hydration status)
Change in Dietary Patterns
Change in Physical Activity and Structured Exercise
Change in Appetite
Baseline vitamin D intake and status and change in serum vitamin D

*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.)*:

Safety Endpoints
Excess weight gain (>1.5 lb/wk)
Change in and absolute values for several health outcomes including blood pressure, serum cholesterol, fasting and 24-hour glucose concentration and hydration status
Excessive muscle soreness and/or pain
Muscle of joint injury
General fatigue

5.0 Study Design and Statistical Analysis Plan

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

This study is a randomly assigned intervention study that will evaluate the effect of a 10-wk diet and exercise regimen designed to promote healthy weight gain in athletes and military personnel (who desire weight gain). This will include increasing energy intake by 500 additional kcal/day (above weight maintenance diet) through daily provision of either peanut-based whole foods/snacks (peanut group) or a similar, high-carbohydrate, peanut-free snack (control group) along with a supervised resistance training regimen.

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 analyzed using IBM SPSS statistics software. Repeated measures ANOVA will be used to assess changes in dependent variables including participant body weight, body composition and anthropometric variables by group and across time (Aim 1). Student t-tests and simple and partial correlation statistics (Pearson Product or Spearman Rank) will be used to evaluate the magnitude of change in body weight/lean mass according to participant characteristics (sex, age, training status, somatotype) and significant adaptive changes in thermogenesis and appetite (Aim 3). Descriptive statistic methodologies will be applied to evaluate the ease and acceptability of peanut foods in the weight gain meal plan (Aim 2). The significance level will be set a priori at $p<0.05$

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.*

The research will be conducted at Virginia Tech. Our research team will identify and recruit potential research participants and also perform the research procedures in the Human Integrative Physiology (HIP) Laboratory in the Garvin Innovation Building or the Nutrition and Exercise Metabolism (NEM) Laboratory in the research building located on 2270 Kraft Drive in the CRC.

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.*

The Research does not involve administration of drugs. The research does involve use of Dual-energy x-ray absorptiometry (DXA/DEXA) scans that will be performed at 4 timepoints during the study

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:

N/A

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:

The medical devices/equipment used in this study include the DXA and the CGM sensors.

The DXA will be used to assess total body composition. Both devices are FDA approved and the research will involve employment of these devices for approved uses. Scans will be performed only by members of the research staff who are trained and certified bone densitometry

technologists (CBDT) through the International Society of Clinical Densitometry.

The CGM will be used only for research and not diagnostic purposes for the intended FDA approved intent of monitoring blood glucose concentration over several days. Prescriptions are not required to obtain CGM devices from Abbott Laboratories (FreeStyle Libre) if they are to be used for research purposes. Dr. Davy has experience using CGM for research purposes as part of ongoing collaborative research projects.

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:

Sixteen men and sixteen women between the ages of 18 and 40 will be randomly assigned to the peanut intervention or control group (8 men/8 women/group). A computer program with a random number generator will be used by the study coordinator to assign male participants and female participants to the peanut or control group (i.e., each sex will be separately randomized).

As outlined in the table, eligible participants will undergo a 10-wk diet and training regimen designed to promote healthy weight gain. This will include increasing energy intake by 500 additional kcal/day (above their typical weight maintenance diet and accounting for energy requirements of resistance training) through provision of peanut-based whole foods/snacks or high-carbohydrate containing control snacks along

with a supervised rigorous resistance training regimen designed to promote muscle hypertrophy. After providing informed consent, participants will be randomly assigned to the peanut or control group based on sex. Outcome data will include assessment of body mass, total and regional body composition via DXA and body anthropometrics, skinfolds and somatotyping using the International Standards for Anthropometric Assessment (ISAK) (Stewart, Marfell-Jones et al. 2011) along with measures of resting metabolic rate (RMR), circulating hormones and serum/plasma biomarkers. The latter includes measurement of potentially important hormones/ biomarkers of anabolism, stress, overfeeding, and cardiometabolic health. Compliance to the nutrition and exercise regimen will be evaluated at regular intervals and include collection of 3-day food records (performed at baseline and 3-days prior to each assessment point) and physical activity data collected by digital technology (Garmin Vena SQ smart watch and My PT Hub app).

Dietary Intervention. The peanut group will be provided with a variety of whole and laboratory-prepared peanut-containing snacks to meet their daily excess energy. Examples of snacks include peanut butter cookies, trail mix, peanut butter banana smoothies, and peanut butter and fruit jam sandwiches that provide 500 kcal (~15 g of protein). The control group will be provided similar 500 kcal high carbohydrate containing snacks (e.g., blueberry muffins, trail mix with chocolate chips and dried fruit, fruit smoothie, etc). A complete list of these snacks is uploaded as supplemental files and will be shown to participants. These snacks will be prepared in the research kitchen in Wallace Hall (Dr. Davy's Laboratory). Participants will be encouraged to consume snacks within an hour of resistance training and/or before bed (Phillips and Van Loon 2011). Participants will also undergo weekly assessments to ensure compliance and adequate progress with excess energy that will be adjusted upward with weight gain to avoid the potential effects of acute energy imbalance due to total body/lean mass gain, and help ensure adequate (~0.5 pound/week) but not excessive (>1.5 pounds/week) weight gain. Dr. Davy has previously completed a similar diet-induced weight gain protocol in non-athletes; This protocol overfed 14 male (Gentile, Orr et al. 2007) and 3 female (unpublished data) participants ~1000 calories per day over baseline requirements using commercially-available food/beverages as the source of excess daily energy. Both investigators are research dietitians and have extensive experience with assessment of energy balance and with partial- and fully-controlled feeding trials.

Resistance Training. Participants will undergo rigorous resistance training three days/week in the NEM laboratory under close supervision. The following will be performed with weight machines (Nautilus) and free weights: leg press, squat, leg extension, leg flexion, toe raise, lat-pull down, dead lift, shoulder press, bench press, bicep curl, triceps extension and abdominal twist/crunch. The overall goal will be to have participants perform 10 sets of exercises that work the major muscle groups each week in accordance with a recently published position stand of the International Universities Strength and Conditioning Association (IUSCA) (Schoenfeld et al 2021) with slightly different set of exercises performed on each of the three days. Participants will begin with light to moderate resistance for 8-15 repetitions, after which resistance will be gradually increased in the next 5 workouts. After that, three to five sets of each exercise will be completed using modified methodology of Bartels et al (Kreider, Klesges et al. 1996) (previously shown to effectively promote weight gain) in accordance with the latest

guidelines for promoting muscle hypertrophy (Schoenfeld et al 2021). . Resistance will be adjusted upward after 8 repetitions are fully executed using proper technique. Participants engaged in a sport or military training activity will be instructed to maintain their normal training throughout the study.

Table 1. Overview of Data Collection				
Overview of Data Collection				
	Baseline	3 wk	7 wk	11 wk
Body Mass (weight)	x	x	x	x
Body Composition by DXA and spot urine sample; Pregnancy test by urine in female participants	x	x	x	x
Skinfolds & circumferences	x			x
Resting metabolic rate-RMR	x	x		x
Blood via venipuncture and Urine vis 24-hr collection				
CBC and TSH	x			
Testosterone (free &total)	x	x	x	x
Growth Hormone	x	x	x	x
Insulin-like growth factor	x	x	x	x
Leptin	x	x	x	x
Insulin	x	x	x	x
Free T4	x	x	x	x
Cortisol	x			x
Ghrelin	x	x	x	x
Urinary creatinine and urinary iodine	x			x
Health outcomes				
Blood Pressure	x	x	x	x
Serum Lipids	x			x
Serum Vitamin D	x			x
Fasting blood glucose	x			x
Continuous glucose monitoring	x			x
Compliance and Fitness Testing				

Strength Testing	x			x
Aerobic Capacity (VO2max) and walking efficiency	x			x
Food Records and Appetite rating for 3-day	x	x	x	x
Vitamin D Food Frequency and Lifestyle Questionnaire	x			
Exercise tracking for 3-days (smart watch)	x	x	x	x
Daily snack tracking logs and exercise logs	←	↔	↔	→
Questionnaire-Snack acceptability & exit survey				x

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.*

Phone Screen

Those who respond to the investigation's advertisements will be scheduled to complete a brief telephone (or HIPAA-compliant Zoom) screening to confirm basic eligibility criteria. Participants will be made fully aware of the eligibility criteria, time commitment, possible risks and their right to withdraw from the study at any time. A phone screening script and phone screening data collection form (uploaded) will be used for phone screening and will conclude with a brief oral diet history performed by a research team member who is also a registered dietitian nutritionist.

Baseline Screening (approximately 45 minutes)

Informed Consent: Participants will be provided an informed consent form following the phone screening and in advance of coming to the laboratory.

Health History: Subjects will be asked to complete a standard health/medical history questionnaire, which will be used to screen for health issues (e.g., coronary or congenital heart disease) or other reasons (medications which influence study results) that would preclude participation (see uploaded Health History Screening Questionnaire). This questionnaire has been used by Dr. Larson-Meyer for clinical studies for the past 17 years.

Physical Activity: Subjects will be asked to complete a questionnaire regarding habitual physical activity and exercise.

Skinfolds: Skinfolds will be measured at specific anatomical sites that include the triceps, subscapular, biceps, iliac crest, supraspinale, abdominale, front thigh, and medial calf using Harpenden skinfold calipers according to ISAK procedures with the sum of skinfolds used as the outcome variable. Anthropometric breadths and girths will be

obtained using a small sliding caliper and a flexible steel tape measure at the epicondylar humerus, epicondylar femur, midarm (relaxed and flexed), waist, hip, and calf. These will be used both as outcome variables and for initial somatotyping at baseline. This will be done by a trained investigator.

Blood Draw: If the participant is able to come into the laboratory after a 12-hour overnight fast, blood will be drawn by venipuncture for the following: a CBC, TSH, testosterone (free and total), growth hormone, insulin-like growth factor, leptin, insulin, thyroid hormone, cortisol, ghrelin, blood glucose, and serum cholesterol. Blood will be collected during the baseline session if the participant is not fasted as outlined below. In total, blood will be draw four times during the study by venipuncture from a vein in the arm. These include baseline, and at 3, 7 and 11 weeks as explained below. Blood will be drawn with the participant seated quietly in a phlebotomy chair. Approximately 20-25 ml will be obtained for screening/baseline.

<u>Baseline Laboratory Testing Session (approximately 2 hours and 30 minutes).</u>
All measurements will be performed in the morning after a 12-hour fast (no caffeine) with the participants instructed not to engage in heavy exercise for 36 hours prior to testing and to adequately hydrate the evening prior. This visit may be split into two shorter visits.

Resting Metabolic Rate (RMR; also called Resting Energy Expenditure): Participants will lay supine on a bed. A clear ventilated hood will be placed over their head, neck and shoulders and plastic drapes will be tucked under them to avoid leakage of air. Air will be pulled thru the hood with a pump and their expired air will be measured for oxygen and carbon dioxide content. The number of calories expended will be calculated using the total volume of air expired and the oxygen and carbon dioxide content. The ratio of carbon dioxide produced to oxygen consumed will be used to estimate carbohydrate and fat utilization.

Blood Pressure: Blood Pressure will be measured in the right arm using an automatic blood pressure monitor.

Blood draws and hormone and metabolite analyses: If blood was not drawn at the screening visit, it will be drawn immediately after the RMR for a CBC and analysis of TSH, testosterone (free and total), growth hormone, insulin-like growth factor, leptin, insulin, thyroid hormone, cortisol, ghrelin, blood glucose and other factors. Blood will be drawn by venipuncture as explained above for screening. Approximately 20-25 ml will be obtained during the screening/baseline visit.

Body Weight Height and Composition: Body weight and height will be measured on a digital physician's scale. Percent body fat and fat-free mass will be measured in all subjects via DXA scan.

Urine sample and pregnancy test: All participants will be required to provide a small cup of urine immediately before the DXA. The urine will be evaluated for hydration status

via specific density using a refractometer. A pregnancy test will be performed on the urine sample for female participants.

Walking Economy and Aerobic capacity (VO2max): A graded exercise test will be performed on a treadmill to assess aerobic fitness via indirect calorimetry (Parvo Medics TrueOne 2400). Heart rate will be measured during the test by a heart rate strap and sensor. The exercise test will consist of walking or running on the treadmill starting at a self-selected pace with the grade or speed increasing every min until the participant reaches volitional exhaustion and can no longer continue. The test will begin with a walking economy test in which the participant walks for 4-minutes at 2.5, 3 and 3.5 mph. The last two minutes of oxygen consumption and carbon dioxide production data will be used to determine metabolic economy (ml oxygen consumed per kg body weight per minute relative to the set work performed). Following cumulation of the standard economy test, the workload will be increased each minute by increasing treadmill speed or grade by 0.5 mph or 2.5%, respectively until the participants reach volitional exhaustion. The entire exercise testing protocol (metabolic economy plus VO2max) will last 20-25 minutes.

Dietary food records, exercise tracking, continuous glucose monitoring (CGM), and 24-hour urine collection: Following laboratory testing, participants will be instructed on completing 3-day food records and exercise tracking, performing the CGM and completing the 24-hour urine collection. Participants will be asked to record all of the food they eat for 3 days, rate feelings of hunger and appetite using standardized appetite rating sheets, and wear a smart watch over this same time period. At baseline only, they will be asked to complete a food-frequency and lifestyle questionnaire specific to vitamin D intake/status simultaneously with the 3-day food records. The smart watch will use an app to measure heart rate and estimate energy expended during structured exercise or physical activity; this will be used to help estimate the participants total energy expenditure. It will be necessary to have the GPS function turned on during the collection of physical activity data but only data related to time, distance or intensity and not GPS coordinates will be downloaded or recorded. CGM will also coincide with the three-day food records using a device called a “Continuous Glucose Monitor” that is typically worn on the back of the upper arm. This requires that a small amount of interstitial fluid (0.5 microliters) be sampled every 15 minutes (96 times per day) throughout the course of the 3-day measurement. In addition, participants will be asked to collect urine for 24 hours during one day of this recall or within the first week of the study. Participants will void and discard the first morning urine sample and then collect all subsequent samples in provided clinical urine jugs for 24 hours, ending with the first sample upon waking the next day. Participants will schedule a time to drop off their urine during one of their resistant training sessions the first week or at a separate time, if it is more convenient. The instructions the participants will receive on using the Smart Watch, using CGM, and collecting the 24-hour urine are uploaded as an Addendum.

Healthy Weight Gain Intervention

Resistance Training: Participants will undergo rigorous resistance training three days per wk under close supervision. The following will be performed with weight

machines (Nautilus) and free weights: leg press, leg squat, leg extension, leg flexion, toe raise, lat-pull down, dead lift, shoulder press, bench press, bicep curl, triceps extension and abdominal twist/crunch. The overall goal will be to have participants perform 10 sets of exercises that work the major muscle groups each week in accordance with a recently published position stand of the International Universities Strength and Conditioning Association (IUSCA) (Schoenfeld et al 2021) with slightly different set of exercises performed on each of the three days. Participants will begin with light to moderate resistance for ~8-15 repetitions, after which resistance will be gradually increased over the following 5 workouts. After that, three to five sets of each exercise will be completed using modified methodology of Bartels et al in accordance with the latest guidelines for promoting muscle hypertrophy (Schoenfeld et al 2021). The resistance training sessions will last approximately 60 to 75 minutes.

Dietary Intervention: The peanut group will be provided with a variety of whole and laboratory-prepared peanut-containing snacks to meet their daily excess energy. The snacks will be calculated to contain 500+/- 10 kcal and 15 grams of protein (using Nutrition Data System for Research nutrient analysis software). A complete list of these snacks is uploaded as supplemental files. The control group will be provided similar 500 kcal high carbohydrate containing snacks that contain ~5 g protein.. Participants will be informed that they are in the peanut or non-peanut group but the dietary composition differences between these snacks will not be mentioned or discussed. Participants will be encouraged to consume snacks within an hour of resistance training and/or before bed (Phillips and Van Loon 2011). Participants in both groups will be allowed to select from several different snack options offered each week. Participants will also undergo weekly assessments during one of the resistance training sessions to ensure compliance and adequate progress with excess energy adjusted upward with weight gain to avoid the potential effects of acute energy imbalance due to total body/lean mass gain and help ensure adequate (~0.5 pounds/week) but not excessive (>1.5 #/week) weight gain.

Daily Snack and Exercise Tracking Logs: Participants will keep daily record of consumption of the provided snacks as well as other exercise training undertaken using a simple snack and exercise tracking log. These logs require participants to place a check mark by snacks consumed and to quickly fill in time spent in other exercise training activities. Logs will be turned in to the researchers every two weeks during resistance training sessions.

Strength and Fitness Testing: Strength of a major upper and lower body group (i.e. bench press and leg press) will be assessed on the Nautilus equipment at the beginning and end (wk 11) of the 10-wk program by recording the maximal weight that can be lifted once correctly. The change in the aforementioned 80% RM value will also be documented for use as data points.

<u>Week 3 Laboratory Testing Session (approximately 75 minutes)</u>
This session will be scheduled at the end of week 3/beginning of week 4 and will be performed in the morning after a 12-hour fast (no caffeine) with the participants instructed not to engage in heavy exercise for 36 hours prior to testing and to adequately

hydrate the evening prior. As outlined in the Table, all tests conducted as part of baseline testing will be performed except for CGM, 24-hour urine collection, walking economy and aerobic capacity. Blood will be drawn by venipuncture from a vein in the arm with the participant seated quietly in a phlebotomy chair. Blood and urine will be analyzed for selected hormones and metabolites (as outlined in the Table). Approximately 15 ml will be obtained for the Week 3 visit.

Week 7 Laboratory Testing Session (approximately 60 minutes)
This session will be scheduled during week 7 and will be performed in the morning after a 12-hour fast (no caffeine) with the participants instructed not to engage in heavy exercise for 36 hours prior to testing and to adequately hydrate the evening prior. As outlined in the Table, all tests conducted as part of baseline testing will be performed except for the RMR, CGM, 24-hour urine collection, walking economy and aerobic capacity. Blood will be collected but analyzed only for selected hormones and metabolites. Blood will be drawn by venipuncture from a vein in the arm with the participant seated quietly in a phlebotomy chair. Blood will be analyzed for selected hormones and metabolites (as outlined in the Table). Approximately 15 ml will be obtained for The Week 7 visit.

Week 11 Final Testing Session (approximately 2 1/2 hours)
This session will be performed as outlined in the Baseline testing section. Blood will be drawn by venipuncture from a vein in the arm with the participant seated quietly in a phlebotomy chair. Blood will be analyzed for all hormones and metabolites analyzed at baseline except the CBC. Approximately 20 ml will be obtained for screening/baseline. Participants will also provide feedback related to the palatability and ease of consumption of the snacks they consumed on a questionnaire and also be asked to complete a brief exit survey.

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*

The following safeguards will be employed to reduce the probability and magnitude of risks associated with study participation. The specific risks are highlighted in Section 17.

Resistance training: Potential risks associated with resistance training include severe muscle soreness, muscle or joint strain or injury or an abrupt elevation of blood pressure. These risks will be minimized by enrolling young healthy participants who have had prior experience with resistance training and, supervising all training sessions (with emphasis on proper form, lifting procedures) and tapering resistance over the first ~2 weeks.

CGM: Potential risks associated with the CGM include discomfort during the insertion, pain, inflammation, redness/rash, swelling, minor bleeding and minor infection at the site. These risks will be minimized by having a trained member of the research staff perform the procedure under aseptic conditions. Participants might also experience the aforementioned 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, participants have the ability to remove the CGM at will. Symptoms typically resolve within a short time (approximately one week).

Questionnaires and Study Logs: All study questionnaires (except the food records, exercise logs and snack logs) will be collected with the participant sitting in a private setting in the laboratory. Questionnaires will be placed in each participant's study file date entered for data analysis.

Blood draws: Blood will be collected using universal precautions by a trained technician. Blood will be drawn by venipuncture from a vein in the arm with the participant resting in a phlebotomy chair. Blood will be drawn at four times during the study (screening/baseline, 3 weeks, 7 weeks and 11 weeks) with a total of 75 ml of blood collected over the course of the 12-13 weeks.

DXA scan: DXA procedure will be performed by trained staff. Participants will be informed of the risk of radiation exposure prior to study enrollment. Female participants will complete a pregnancy test by urine immediately before the DXA.

VO2max/Aerobic capacity: Trained research personnel will be present during the test to correctly place the mouthpiece, monitor all variables during the test and support the participant at the end of the test.

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:

Anthropometric, basic demographic (sex, age), RMR, and exercise testing data will be recorded on data sheets and manually entered into a database (excel format) on a secure computer as outlined in section 8.2 above. Select data (DXA results, RMR, VO2max) may be transferred electronically directly from the DXA or metabolic cart into excel spread sheets if possible. Blood and urine results will be entered directly from laboratory sheets provided by a commercial laboratory or the Metabolic Core at Virginia Tech. Heart rate, energy expended and exercises performed will be downloaded onto the laboratory computer from the Smart Watch. Glucose concentration in interstitial fluid samples by time will be downloaded directly from the CGM sensor onto the lab computer. The majority of lab collection sheets are uploaded with this application.

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. Larson-Meyer's laboratory which will also be locked to only authorized personnel. 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.

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

Blood and urine samples will be stored in a -80-degree freezer in the HIP laboratory currently located in the Garvin Building. Samples will be labeled with the participants' study code (see section 9.4 below), the visit number and the date and time of the collection. No identifying information will be written on specimen samples. The freezer is located in locked room/laboratory.

Blood analyzed by the Metabolic Core at Virginia Tech, housed in the Integrated Life Sciences Building, may also be temporarily stored in a freezer in this laboratory immediately before, during or after analysis.

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:

Some de-identified blood and urine samples will be sent to a commercial laboratory for analysis. There are currently no plans to release data outside of the research team.

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:

Study Codes using a combination of letters and numbers will be used to de-identify subjects from their personal information. No obvious identifiers will be stored with the data; the data spreadsheet, however will include each participants age, sex and starting weight as part of the de-identified data. Original de-identified data collection sheets will be stored in a locked file cabinet as part of study records; scans of some de-identified information may be kept in a password-protected electronic file that is accessible only to research personnel. During the active phase of the study, a master document (key) that will contain the participants name, assigned study code and randomization order will be kept in a password-secured file that will be accessible only to the PI, co-investigator, study coordinator (EM) and doctoral student in charge of the study (Allison Sanchez). The key will be destroyed 6 to 12 months after collection of data from the last participant. De-identified data may be kept indefinitely. Blood and urine samples will be destroyed after 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 participants' involvement in the study ends, interested participants will be provided with individual results related to their body composition, fitness, resting energy expenditure and pertinent blood markers (blood sugar, serum cholesterol, TSH, etc). These data will be summarized on a TBD summary document that the participant can pick up at the lab or have (upon request) mailed to them at a provided address. This form will be submitted as an Addendum before it is provided to the first participant. Participants will only be provided results during the study if it is determined that the participant has a result for any measured outcomes that is out of the normal range; in this case the participant would be provided information about the value and asked to see their personal health care provider. The participants will also be notified when a summary of the study findings is published if an active email address is on file.

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 to 13 weeks, which will include a Zoom or telephone call for screening, a short screening visit in the laboratory, a baseline visit, follow up visits at 3, 7 and 11 weeks, and 10 weeks of supervised resistance training, three times per week. The actual time and frequency of the subject's visits will depend on their schedule and that of the study staff. Participants will begin the study on a rolling basis, but the entire study will take place across approximately one year as dictated by the funding agency. The investigators will complete primary data analyses within the following year but all analyses of study data may not occur for up to ten years following study completion.

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 telephone (or Zoom) screening to confirm basic eligibility criteria. Participants will be made fully aware of the eligibility criteria, time commitment, possible risks and their right to withdraw from the study at any time. A phone screening form (uploaded) will be used for this purpose and will conclude with a brief oral diet history performed by a research team member who is also a registered dietitian nutritionist (RDN).

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):

To be eligible, participants must be in good overall health with the desire to gain at least 5 pounds of body weight to enhance effectiveness in sport, fitness, or military training/competition. Participants of all body sizes will be allowed to participate as long as they meet the aforementioned criteria (desiring weight gain for sport, fitness or military/training/competition); this will very likely involve enrolling some athletic women or military women who weigh less than 110 pounds at study start. Participants will not be considered if they smoke, are pregnant, have coronary heart disease, have hypertension (on blood pressure medications), have medical and/or orthopedic limitations that might affect the ability to fully participate in the study, are currently taking any dietary supplements or prescribed pharmacological agents that might affect lean tissue accretion, have a current or past history of anabolic steroid use, have a history of or current signs of disordered eating (score >20 on the Eating Attitudes Test), or have an allergy to peanuts and/or tree nuts. Participants will also be excluded if they have an abnormal TSH or hemoglobin (obtained at screening), have high blood pressure (>140/90 mmHg), abnormal serum lipids (e.g. fasting triglycerides <150 mg/dL and total cholesterol <200 mg/dL), have not previously participated in strength/resistance training as part of athletic or military training within the last year or are currently not involved in sport, or military or strength/resistance training at the recreational, high school, collegiate or elite level. Prior to enrollment, detailed diet and exercise data will be collected via a diet history to ensure participants are consuming adequate energy (relative to estimated energy expenditure) and sufficient protein (>1.2 g/kg) from a balanced diet at baseline. The Eating Attitudes Test will be used to screen for disordered eating patterns and help ensure that participant desire to gain weight is not due to general undereating, unhealthy eating patterns or disordered eating. Individuals who score >20 will be encouraged to follow up with their health care provider and provided a list of Registered Dietitians in the Area who specialize in the treatment of disordered eating.

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*

None of the above will participate.
Pregnant women are excluded both because their inclusion would interfere with our study results (i.e., weight gain during pregnancy is different than what we are studying) and because vigorous strength/resistance training with the intent of lean body gains for enhanced sport or military performance is not appropriate during pregnancy.

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 students and employees of Virginia Tech. However, during the consenting process, the participants will be made aware that only members of the research study team 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. If Virginia Tech athletes are interested in the study, they will only be allowed to participate during their off-season and after first obtaining approval from the athletics department.

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):

The sample target of 16 men and 16 women was selected because we believed it would be feasible to recruit and study this number within the time frame and budget set by the funding agency. We, however, will target recruitment of up to 40 participants. Based on limited previous studies in men who have used a range of 12 to 46 subjects, we also believe this number is sufficient to show measurable results.

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:

We anticipate that we may need to screen between 100 and 150 participants to recruit and complete the required 32 participants.

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

20 participants (~10 men/10 women) will be assigned to the peanut-containing snack group and 20 participants (~10 men/10 women) will be assigned to the control snack group as previously mentioned.

15.0 Recruitment Methods

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

Participants will be recruited through flyers placed at strategic locations (gyms, fitness, military and recreational centers, etc.), at colleges and universities in the New River Valley (including Virginia Tech) and through targeted listservs (VT News), emails, and social media posts.

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 will recruit from the general population of athletes and active individuals and those involved with and enlisted in the military. This will include recruiting members of local gyms and fitness centers.

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

As mentioned previously above (15.2), we will identify participants through use of flyers placed at strategic locations and through targeted listservs, emails and social media posts.

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.*

A draft copy of our recruitment flyer is uploaded. This flyer will be posted at strategic locations throughout Blacksburg and the surrounding area. We will seek permission at each site as necessary before posting or hanging a flyer. We also plan to use this same advertisement for emails and a modified version for social media posts (that will be submitted for approval at a later date as an Addendum). Emails will use the subject line "Are you interested in participating in a research study on weight gain to enhance effectiveness in sport, fitness or military training?". Participants will be compensated $10 for completing the baseline visit (not screening visit), $20 for completing the 3-week visit, and $20 for completing the 7-week visit. Participants will be compensated an additional $150 at study end for completing a minimum of 85% of the 10-week training sessions and the final (11-week) visit. This is a total of $200 compensation for completion of all testing visits and at least 85% of the training sessions. Payments will be scheduled after each individual participant completes each of the above visits. Active duty can only accept compensation for blood draws (up to $50 per draw).

16.0 Withdrawal of Subjects

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

Participants could be withdrawn from the study if they are not showing up for appointments and/or training sessions, are not consuming the provided snacks, or are not completing or complying with all procedures. They also may be withdrawn if they develop and injury or illness that would prevent

them from doing everything that is expected for the study or which might compromise their health.

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 not complying with the study, the PI or study coordinator will first discuss these difficulties with the participant and explain the importance of adhering to the intervention for the purpose of the study. If it is determined that the participant be terminated or discontinued from the study for reasons as described above, the PI will mitigate issues leading to these problems. The participant will be provided any information which is available to them (baseline body composition, fitness testing, resting metabolic rate). 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):

Any participant can discontinue participation at any point without consequence.

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)*

Resting Metabolic Rate: Risk of claustrophobia or feelings of anxiety during testing when the clear ventilated hood is placed over their head. To minimize this risk, participants will be screened for claustrophobia and monitored for signs and symptoms that can include sweating, shakiness, tachycardia, increased or rapid breathing, nausea, light-headedness, fainting and fear of actual imminent physical harm. An investigator will monitor this test to ensure participant safety.

DXA Scan: The amount of radiation that subjects will receive in the DXA exam is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount subjects will receive at each scan 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.

Blood draws: Slight discomfort may be expected during blood draws. Risk of developing a small bruise or blood clot in the vein, risk of fainting or dizziness, risk of infection and risk of bleeding are also possible. Universal Precautions will be followed for collection, handling, processing, and disposal of items that may have come into contact with bodily fluids during the collection of blood. Blood draws will be performed by a research phlebotomist (J Rinehart) trained and experienced in the blood draw procedure and in handling minor emergencies such as dizziness and fainting. In case of an emergency, 911 will be called.

Walking Economy and Exercise Capacity (VO2max): There is a small risk of orthopedic injury, treadmill falls or cardiovascular complications that could require a participant to go to the hospital. This includes a heart attack, or even death. In studies involving people with heart disease, the risk of hospitalization was 1 in 500 tests <0.20%). The risk of heart attack was 1 in 2,500 tests (0.04%) and death, 1 in 10,000 tests (0.01%). The risks are likely to be lower in young, healthy subjects who are involved in athletic training and/or exercise. Only experienced staff members will conduct these tests and subjects will be monitored throughout the test for signs of problems based on standards of the American College of Sports Medicine (ACSM). There is a possibility some subjects will be tired after this test and could have sore muscles for a few days.

24-hour urine collection: There are no risks for urine collections. However, 24-hour urine collections may present some inconvenience and/or embarrassment. The inconvenience will be minimized by the provision of a reusable shopping bag to make carrying and storage discrete. Also, female participants will be provided with a female urination devise to make this collection easier.

Continuous Glucose Monitoring. The placement of the device will require that a sensor is inserted into the back of the participant's upper arm. Placement of the sensor may induce some pain during the insertion, inflammation, redness, swelling, minor bleeding and/or minor infection at the site. This will all be minimized by having a trained individual perform the procedure which will take place in aseptic conditions. There is also a possibility a participant may experience these symptoms as a result of contact between the adhesive pad of the sensor and the skin; allergic reactions can also develop in response to the adhesive used to keep the device in place.
If any of these symptoms occur, the participant will be informed that he/she has the ability to remove the CGM and these issues will clear up within a short time period.

Dietary Intervention: There are no real risk to consuming the intervention snacks. The peanut group will be provided with a variety of whole and laboratory-prepared peanut-containing snacks to meet their daily excess energy target of ~500 kcal whereas the control group will be provided a variety of high-carbohydrate snacks. There is a very small chance a participant might develop an allergy or intolerance to one of the snacks. To help avoid this issue, we will screen for a history of food allergies and intolerances including allergies to legumes, peanuts and tree nuts, as well as eggs, dairy, gluten and fermentable carbohydrates.

Resistance (Weight) Training: This project will use standard resistance training procedures with Nautilus equipment that follows ACSM and IUSCA guidelines. Major health organizations recommend resistance training for all age groups, and have found it to be safe with low risk of injury. The most likely possible risk includes muscle soreness. Other possible risks include muscle or joint injury or an abrupt elevation of blood pressure. Our program will recruit only participants who have previous experience with resistance training. The resistance training sessions will be supervised by ACSM-certified personal trainers and emphasize proper form, lifting procedures, and breathing techniques. The program will also be tapered upward over the first ~2 weeks to help prevent excessive muscle soreness and muscle or joint strain. Increases in blood pressure are also unlikely in the population studied. The participants will be informed to contact the PI or any member of the research team in the case of any injury or excessive joint or muscle strain.

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.)
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.)

Included in Section 17.1

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:

It is possible that participants could develop severe muscle soreness by participating in our rigorous resistance training regimen. It is also possible an unknown allergy to peanuts could be identified during the study. These events, however, are not likely. To minimize the potential, we will slowly increase the resistance (weight) used during training over the first 2 weeks and are also recruiting participants who have some previous history of strength/resistance training. A previously known allergy to legumes, nuts or tree nuts is an exclusion criterion for the study.

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

There is minimal risk if a female participant were to become pregnant during the 10 weeks of the study. The most recent committee opinion of the American College of Obstetricians and Gynecologists (April 2020) states that physical activity and exercise (including resistance exercise) in pregnancy are associated with minimal risks and have been shown to benefit most women. Pregnancy tests will be performed at four timepoints during the study (specifically before each DXA), to ensure the participant is not pregnant. Participation in the study would end if it were determined the participant had 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:

Participants will gain information about their body composition, resting metabolism, fitness, and general health. They will also learn about healthy weight gain regimens and how to properly perform strength/resistance training exercises.

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

N/A

19.0 Data Management and Confidentiality

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

Drs. Larson-Meyer and Davy have extensive experience performing data collection on all of the procedures outlined in this proposal as does the study coordinator, Dr. Elaina Marinik. They will ensure all study personnel, including graduate students, will be properly trained to perform all procedures according to standard protocol. Specific quality control measures will be employed to ensure valid indirect calorimetry data are collected during the RMR, walking economy and VO2max tests; These standards are included on data collection sheets for use by members of the study team.

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.:

We will do everything that we can to make sure that study records are kept private. Each participant will be assigned a unique participant code as explained in section 9.4. All data recording sheets and spread sheets will use the subjects' study code. They will not contain the participants' name or date of birth. These will be compiled in a patient research file/chart and stored in a locked file cabinet organized by their unique study code. Their name will be listed only on the phone screening log (which is to be blackened out after they are assigned a participant code), informed consent and on a master participant list that includes the randomization key. The master participant list will be kept in a separate electronic file than the data files; both will be password-protected. The study consents will be kept together in a separate file in a separate location in a locked office. Only authorized study personnel will have access to study data. Results of the study may be published and/or presented at professional conferences. The participants' name or other personal information that would identify them will not be used. All blood collected and post-processed serum and plasma samples will be labeled with the participants unique study code (plus the study visit and date and time of sample collection) and stored in a secure freezer in a locked laboratory until analysis as mentioned in section 9.2. Archives may be kept for up to five years following study analysis. Training of study personnel including graduate students on procedures to ensure secure collection and storage of study data will occur before study initiation.

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?*

Telephone screening forms (that contain participants' names) and the Eating Attitudes Tests will be shredded immediately after all study participants are recruited.Personal information, primary and secondary endpoints and safety data will be kept indefinitely in a secured electronic location by the PI and co-investigator. Personal information will be kept in a separate file than de-identified data. Blood and urine samples labeled with the patients' unique study code may be stored in a laboratory freezer in a locked laboratory for up to five years following the completion of the analyses; only authorized study personnel will have access to freezer samples. The PI will be responsible for transmission of all data or achieved specimens. Although is not anticipated that any data will need to be transported or shared, this would be done only using de-identified data with samples sent using a secure mechanism.

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):

To ensure privacy interests of all interested and enrolled participants, only the minimal amount of personal information and health history will be obtained using a standard health history form; this form has been used by Dr. Larson-Meyer for the past 17 years) This data will be kept in participant files labeled with only the participants' study code in a secured file in a locked room. The data for all participants who do not participate in the study will be destroyed by shredding. The data for participants who do enroll will be entered into an electronic data base using the participants assigned unique study code. Any and all original data collection sheets with the participants' name or identifying information will also be destroyed following entry into the database.

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 questionnaires and anthropometric testing will be performed by trained research personnel in a private setting. Assignment of same-sex researchers will be employed if necessary; however, all study personnel will be trained to exhibit professional behavior and sensitivity when collecting personal health or medical data or when performing body composition or other testing.

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 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) and research staff along with prompt reporting of excessive adverse events and any serious adverse events (AEs) to the Institutional Review Board. All serious AEs will be reported by the PI within 48 hours of occurrence to the IRB and the sponsor.

The safety data monitored will include data related to the blood collections, exercise testing and the supervised resistance training sessions. Specific safety data include any reports of pain, excess swelling, redness or bruising after the blood draws at the needle insertion site, feelings of light headedness, chest tightness or pain or fatigue on exertion during exercise testing procedures, and symptoms of muscle soreness, joint pain, stiffness or unexpected events/issues during the 12 weeks of resistance training. Data will be collected and documented in the participant's chart if a situation arises or when observed by a member of the research team or reported by a participant during a study visits or during supervised resistance training sessions using a general TBD incident reporting form. Safety data will also include excessive changes in blood pressure or weight gain that will be measured during each scheduled study visit, and changes in blood glucose or serum cholesterol that will measured at the beginning and end of the study.

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.

Safety Data collection will start when the first participant is screened and enrolled. The study team will be informed to discuss any observed or reported unusual, excessive or unexpected events immediately with the PI. The PI and/or study coordinator will review study charts and ongoing data collected on all participants on a weekly basis to ensure

safety. In our small study, it is unlikely that use of statistics would be necessary to determine if excessive events were occurring; however, paired t-tests could be used if appropriate (i.e. for blood pressure, blood glucose or serum cholesterol data). We do not anticipate that there would be any specific events, other than the unexpected, that would trigger the suspension of our study,

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:

Participants will not be provided any form of compensation for medical treatment or other damages (for example lost wages, time lost from work, etc.). If a participant becomes injured or sick from the research, they will be referred to a clinic or to their personal health care provider. Medical treatment may be provided at their expense or at the expense of their insurance company.

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:

I do not believe this applies

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:

The participant will be responsible for costs that may include purchase of athletic clothes or shoes to participate in the resistance training sessions or the uncompensated cost that might include transportation, missed work or childcare.

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*

Participants will be first screened over the phone. The phone screening will include an overview of the study, the time commitment and the possible risks to participation. After explaining the study and before conducting the phone screen, a study team member will describe the purpose of the phone screen and what type of data will be collected, and then ask that the participant provide verbal permission to conduct the phone screening. They will also be informed that they may refuse to answer any and all questions. An informed consent form will be emailed (or mailed) to the participant following the phone screening and at least 24 hours in advance of coming to the laboratory for the screening visit.

At the start of the screening visit, a team member will provide potential participants with a written copy of the study consent form and review the document with the participant. Participants will be encouraged to ask questions and seek clarification during the phone screening. The participant will then be encouraged to ask questions before providing written consent. As much time as necessary will be devoted to address participant concerns. Once the participant is ready to sign, he/she will be

allowed to sign in a private room near the door where they may also exit the lab if they no longer wish to participate. Screening and informed consent will be performed by the study coordinator or the doctoral student in charge of the study (A. Sanchez). Participants will be encouraged to ask questions and seek clarification during the phone screening and before signing the consent at the beginning of the laboratory screening visit.

These steps including time to review the consent before the screening visit, time with study staff to review the protocol and address concerns, and time to sign the consent in a private setting and close to a laboratory exit will help minimize the possibility of coercion or undue influence.

To help gauge the participant's understanding, the team member will ask the participant to explain the study, how often they will be asked to consume the peanut or high-carbohydrate snacks, how many times a week they would need to come to the lab to resistance train and how many lab visits are expected.

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 speakers will not be recruited for the study.

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:

Individuals who respond to the advertisements will be contacted by phone where they will be informed of the general plan of the study and all specific procedures included in the study (previously outlined in section 12). Participants will then be given a chance to ask questions regarding study procedures and risks. Those still interested will be screened over the phone to determine eligibility based on desire to gain weight, current exercise history, dietary restrictions, medication use, history of food allergy, and other criteria outlined in section 12.2 (see uploaded Screening form). Eligible individuals will be sent a copy of the consent form via email to review prior to coming to the lab. They will then be given a chance to ask any questions either by email or during their scheduled screening/baseline visit. Those still interested will be asked to sign the consent during their first visit, before any data is collected. This information is detailed in section 24 above. A copy of the informed consent will be sent to all participants.

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

Waiver of written consent to perform the phone screening is requested. The phone screening form has been uploaded.

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:

See the attached participant consent form

26.0 Resources Available

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

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

The PI is a Professor in the Department of Human Nutrition, Foods and Exercise at Virginia Tech. She currently has a 33% research appointment and will oversee four doctoral students in the fall. She has previously served as a research dietitian and research exercise scientist at the National Institute of Diabetes & Digestive & Kidney Diseases in Phoenix and the Pennington Biomedical Research Center in Baton Rouge, LA, respectively, and has experience conducting exercise training studies and controlled feeding trials. The PI and Co-investigator will dedicate time to this study as well as the study coordinator, Dr. Marinik, and two doctoral students, Jake Reynolds and Allison Sanchez. Jake is an ACSM certified personal fitness trainer and Allison is an Army officer and registered dietitian nutritionist or RDN. The investigators have used the following laboratories at Virginia Tech where the research will take place. In addition, the Department has secured space for Dr. Larson-Meyer's NEM Laboratory on Kraft Drive close to the HIP Laboratory.

The Laboratory for Eating Behaviors and Weight Management (Director: B. Davy) is located in Wallace Hall and encompasses a ~600 sq ft Metabolic Kitchen with a ~900 sq ft research Dining Laboratory area and a research dietitian computer workstation (for dietary analysis software), reach-in freezer, and refrigerator for storing meals to be consumed off-site, and an additional ~250 sq ft space housing stadiometers, scales, tables for completing questionnaires, a private room for measuring blood pressure and anthropometrics, and a file storage area.

The Human Integrative Physiology Laboratory is housed in both Wallace Hall (Body Composition Laboratory, Resting and Exercise Testing Laboratory (DE Larson-Meyer)) and The Garvin Innovation Center (Exercise training equipment) located within a 5 min-drive from Wallace Hall. Major equipment items in the Body Composition and Resting and Exercise Testing Laboratories include two DXAs, Harpenden skinfold calipers, anthropometric tape measures, Parvomedics TrueOne 2400 metabolic cart, hospital bed, reclining phlebotomy chairs, Woodway treadmill, and blood pressure monitors. Additional research space is also available in Wallace Hall for sample processing (i.e., wet lab areas) and storage (-80 freezers). The Garvin space houses the 7-piece Nautilus equipment, weight bench and two dumbbell sets in a 650 sq ft room with ample space for training, and free parking for participants. Restrooms, showers and changing facilities are also available.

The Metabolic Core at Virginia Tech will perform the majority of the biochemical analyses. This core laboratory is housed in the Integrated Life Sciences building and includes a 140 sq ft. laboratory space dedicated to biochemical assays. This Core laboratory has a BioTek Synergy 2, a multi-mode microplate reader equipped with Gen5 software capable of measurements of absorbance utilizing a monochrometer for wavelength selection from 200nm to 999nnm, and for fluorescence with excitation and emission filters for luminescence using a liquid-filled light guide

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