22-168 The Effect of Impact Loading on Bone Biomarkers in Energy-Restricted Female Runners

INSTRUCTIONS:

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

PROTOCOL TITLE:

Include the full protocol title.

The Effect of Impact Loading on Bone Biomarkers in Energy-Restricted Female Runners

PROTOCOL NUMBER:

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

22-168

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

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	3/1/2022	Clarified time commitment for subject participation and payment structure in the protocol and consent form (Sections 1.0, 15.4, 17.4). Added more details regarding future undergraduate study personnel in section 26.	No
2	3/16/2022	Increased compensation to $100 split into two payments of $25 and $75. (Section 15.4) Clarified exclusion criteria: no contraceptive use, no recent (within 12 mo) or current eating disorder (Section 12.2) Expanded on protocol justification (Section 3.3) Clarified running time of protocol (Section 8.1)	Yes
3	3/29/2022	Added pregnancy test after the 3-week washout period on day 1 of the second experimental condition (Sections 8.1, 8.2, 12.3, 17.4)	Yes
4	5/20/2022	Protocol for impact loading exercise sessions modified slightly to include recovery period (Section 8.2) Additional assays (leptin, cortisol, ferritin, 25(OH)D, Nesfatin-1) (Section 4.1 and Table 1 in Section 8) Added vitamin D questionnaire at baseline (Section 8.2) Change to weight safety endpoint (Section 4.2 and justification in section 7.2) Change timepoints for running economy Added menstrual cycle question to phone screening form	Yes
5	6/24/2022	Baseline DXA moved from visit 2 to visit 1 (Section 8.2)	Yes

		Added a mealtime log during the 5-day experimental phases (Section 8.2) Protocol for treadmill run decreased to 65%VO2max (previously 70%VO2max) (Section 8.2)	
6	9/6/2022	Inclusion criteria adjusted to include participants ages 18-35 (previously 18-30) and removed running requirement of 30 miles/week and VO2max criteria (Section 12) Additional iron status markers (TIBC and serum iron) (Section 4.1, Table 1 Section 8) Exclusion criteria: criteria for low BMD adjusted to z-score <-2 (Section 12)	Yes
7	7/10/2023	Inclusion criteria adjusted to include participants with a BMI 18.5-30 (Section 12)	Yes
8	9/8/23	Increase sample size to 20	No

Table of Contents

1.0 Study Summary

Study Title	The Effect of Impact Loading on Bone Biomarkers in Energy-Restricted Female Runners
Study Design	Randomized Cross-Over Intervention Study
Primary Objective	Determine the effect of short-term, high-impact loading on biomarkers of bone remodeling in trained, female long-distance runners in the presence of low energy availability.
Secondary Objective(s)	Exploratory Aim: Examine the relationship between undercarboxylated osteocalcin and impact loading on glucose metabolism during acute low energy availability.
Study Population	Eumenorrheic, female long-distance runners who do not have existing low energy availability.
Sample Size	20
Research Intervention(s)/ Investigational Agent(s)	Research volunteers will undergo two experimental conditions of low energy availability in a randomized order: Low energy availability with daily running only (RUN) and low energy availability with daily running and impact loading (RUN+IL). Each 5-day experimental condition will be initiated in the follicular phase and separated by a washout period of one menstrual cycle (approximately 24 days).
Study Duration for Individual Participants	19 days of intervention and study visits including one screening visit, one baseline testing visit, 3 days of tracking normal diet and exercise, and two experimental conditions of 7 days separated by a 3-week washout period of normal diet and exercise
Acronyms and Definitions	ACSM, American College of Sports Medicine; BMD, bone mineral density; CBC, complete blood count; CGM, continuous glucose monitoring; CTX, C-terminal telopeptide of type 1 collagen; DXA, Dual-Energy X-ray Absorptiometry; HIP Laboratory, Human Integrated Physiology Laboratory; LEA, low energy availability; NEM Laboratory, Nutrition and Exercise Metabolism Laboratory; P1NP, N-terminal propeptide of type 1 procollagen; Trap5b, tartrate-resistant acid phosphatase; TSH, thyroid-stimulating hormone; unOC, undercarboxylated osteocalcin; VO2 max, maximal oxygen uptake during exercise, also known as aerobic capacity;

2.0 Objectives

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

1) Determine the effect of short-term, high-impact loading on biomarkers of bone remodeling in energy-restricted, female long-distance runners.
2) Examine the relationship between undercarboxylated osteocalcin and impact loading on glucose metabolism during acute low energy availability.

2.2 State the hypotheses to be tested:

The addition of 50 high-intensity impact loading jumping exercises per day to usual run training will result in less suppression of bone formation following 5 days of endurance running in an energy-restricted condition compared to daily running in an energy-restricted state without high-impact loading exercises.

3.0 Background

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

Maintaining adequate energy intake is essential for athletic performance and overall general health. Yet, many athletes experience low energy availability (LEA) by failing to consume enough calories to meet their energy demands. Energy availability is the amount of energy remaining from dietary energy intake (EI) to support general health and bodily functions after accounting for exercise energy expenditure (EEE), and is commonly expressed relative to fat-free mass as kilocalories per kilogram of fat-free mass (FFM) per day (kcal/kgFFM/d). According to the Life History Theory, the human body will adapt to conserve energy under conditions of biological stress, such as energy-restriction and LEA, by downregulating biological processes that are less essential for immediate survival (Shirley, Long et al. 2022). The performance and health consequences of LEA are characterized by the syndrome known as Relative Energy Deficiency in Sport (RED-S) (Mountjoy, Burke et al. 2018). Specific to this proposed study, athletes suffering from RED-S and chronic LEA may experience several inter-related health consequences involving endocrine function, reproduction, and bone metabolism. Previous studies indicate LEA is associated with increased risk for bone stress injuries and low bone mineral density (BMD) (Tenforde, Carlson et al. 2016, Tenforde, Carlson et al. 2018, Gibbsm Battuv et ak, 2014), in addition to suppression of certain bone-regulating hormones including estrogen, insulin, vitamin D, triiodothyronine (T3), and insulin-like growth factor (IGF-1) (McCall, Ackerman 2019).

Previous Studies on LEA and Biomarkers of Bone Metabolism. Biomarkers of bone formation and resorption are often used as surrogate markers for assessing bone metabolism in the short term given that it can take months or even years for changes in bone microarchitecture and BMD to manifest. In a study of 8 male distance runners,

serum N-terminal pro-peptide of type 1 collagen (P1NP), a marker of bone formation, and IGF-1 decreased by 15% and 17%, respectively, after 3 consecutive days of treadmill running with energy intake restricted to 50% of estimated needs (Zanker and Swaine 2000). In a more recent cross-over design study (Papageorgiou, Elliott-Sale et al. 2017), changes bone biomarkers were measured in 11 women and 11 men in response to 5 days of daily running under optimal EA conditions (45 kcal/kgFFM/d) and 5 days of daily running under low EA conditions (15 kcal/kgFFM/d). In both conditions, participants ran on a treadmill until an energy expenditure of 15 kcal/kgFFM was achieved. Diets provided 60 kcal/kgFFM/d and 30 kcal/kgFFM/d for the optimal and low EA conditions, respectively. In the female participants, P1NP was significantly lower after the low EA condition compared to optimal EA. Additionally, ß-carboxyl-terminal cross-linked telopeptide of type 1 procollagen (ß-CTX), a marker of bone resorption, increased 19% in response to the low EA condition and this increase was significantly different from the change in ß-CTX observed in the optimal EA condition. No significant change in P1NP or ß-CTX were observed in men. This suggests women may be more sensitive to the effects of LEA on bone metabolism and, therefore, be at greater risk. In another cross-over design study of 10 female runners, changes in P1NP and ß-CTX were measured after completion of three, 3-day experimental conditions: optimal EA (45 kcal/kgFFM/d), LEA achieved through dietary restriction only (15 kcal/kgFFM/d), and LEA achieved through a combination of dietary restriction and daily running (15 kcal/kgFFM/d) (Papageorgiou, Martin et al. 2018). In the combined diet and exercise LEA condition, participants received diets providing 30 kcal/kgFFM/d and expended 15 kcal/kgFFM/d during a treadmill run. This study found P1NP was significantly reduced after 3 days of LEA achieved through diet only, but not after the combined LEA condition with running. These findings suggest the negative effects of LEA on bone metabolism may be counteracted or masked by the osteogenic effects of weight-bearing exercise, such as running. There were no significant changes observed in ß-CTX in response to either LEA condition. Based on these short-term studies, it appears suppression of bone formation occurs before measurable increases in bone resorption. Additionally, the severity of bone impairments may depend on the degree of energy restriction given the dose-response relationship observed between LEA and select bone turnover markers in exercising women (Ihle and Loucks 2004).

Benefit of High-Impact Loading on Bone Metabolism. High-impact loading exercises such as jumping, bounding, and plyometric training place a high level of mechanical strain on bone and can elicit osteogenic adaptations (Hutson, O'Donnell et al. 2021). There have been a limited number of studies showing mixed results on the short-term effects of high-impact loading on bone biomarkers (Rantalainen, Heinonen et al. 2009, Rogers, Dawson et al. 2011). Additionally, very few studies have included energy intake assessments and most studies have been conducted in non-athletes. In one study of 26 female non-athletes, markers of bone formation (osteocalcin and bone specific alkaline phosphatase (BAP)) and bone resorption (tartrate-resistant acid phosphatase (TRAP5b) and CTX) were measured in response to either a control condition or jumping intervention (Kishimoto, Lynch et al. 2012). Volunteers in the jump group performed 10 jumping exercise per day at a frequency of 5 times a week for 2 weeks. Bone resorption measured by CTX was lower in the jump group compared to baseline. Additionally, TRAP5b was significantly lower in the jump group compared to the control group,

however, there was no significant changes in TRAP5b from baseline in either group. Interestingly, the jump intervention significantly lowered BAP, a marker of bone formation. No changes were observed in osteocalcin within or between groups. This study was limited based on lack of energy intake assessment and the potential for inter-subject variability in bone response to jumping exercises. Despite the theoretical basis for high-impact exercise as a countermeasure for bone resorption during LEA, there have been no controlled trials to date that have investigated this theory in energy-restricted athletes.

Undercarboxylated Osteocalcin (unOC) and Glucose Metabolism. This study seeks to contribute to understanding the potential association between osteocalcin in glucose metabolism in humans. Undercaboxylated osteocalcin (unOC) is the active form of osteocalcin and is released in response to osteoclast activity during bone resorption (Moser and van der Eerden 2019). The role of unOC to regulate glucose metabolism in humans is unclear (Lin, Brennan-Speranza et al. 2018). In a cross-sectional study, unOC was found to be associated with insulin secretion and sensitivity in lean male patients (BMI <25 kg/m^2) (Fernández-Real, Izquierdo et al. 2009). Subjects in this study were generally healthy and non-athletes. In a meta-analysis of osteocalcin and glucose metabolism, a weak negative correlation ($r=-0.09$, $p<0.5$) was found between unOC and fasting plasma glucose in women (Liu, Guo et al. 2015). However, the association between unOC and glucose metabolism was found to be stronger in men than in women. There is limited data on the effects of LEA on glucose metabolism in athletes without diabetes. One study of 7 male long-distance runners found muscle glycogen was reduced by approximately 30% after 3 days of endurance training under energy-restricted conditions (EA = 18.9 ± 1.9 kcal/kgFFM/d) (Kojima, Ishibashi et al. 2020). No studies to date have investigated the relationship between undercarboxylated osteocalcin and glucose metabolism in energy-restricted athletes.

3.2 Describe any relevant preliminary data:

N/A

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

Maintaining adequate energy intake is essential for athletic performance and general health. Yet, many athletes experience low energy availability (LEA) by failing to consume enough calories to meet their energy demands. Recent studies estimate that the prevalence of LEA in athletes ranges from 22% to 58% with a high prevalence in women and endurance sports (Logue, Madigan et al. 2020). Chronic LEA is associated with several inter-related health consequences involving endocrine function, reproduction, and bone metabolism. Impaired bone health is one of the most concerning consequences of chronic energy restriction. Left untreated, prolonged energy deficiency may impair bone accrual during

adolescence and bone formation in adulthood, leading to an elevated risk of fracture and osteoporosis later in life (Papageorgiou, Dolan et al. 2018).

The recommended treatment for LEA is to increase energy availability to optimal levels (45 kcal/kgFFM/d) by increasing EI, decreasing EEE, or a combination of the two (Kuikman, Mountjoy et al. 2021). However, inadequate energy is not always intentional as in the cases of disordered eating/eating disorders and "cutting weight". Some athletes may experience LEA unintentionally due to factors such as inadequate knowledge of fueling recommendations, decreased appetite, lack of time, or low food security (Wasserfurth, Palmowski et al. 2020). Therefore, not all athletes may be willing or able to achieve optimal energy availability. Given the significant risk to long-term bone health, strategies to counteract the effects of LEA on bone metabolism are necessary.

Compared to inactive controls, runners on average have higher BMD and bone strength (Scofield, Hecht et al. 2012). However, lower total and site-specific BMD has been reported in female endurance runners compared to sprinters and athletes competing in higher impact sports (Tenforde, Carlson et al. 2018, Mudd, Fornetti et al. 2007). Lower BMD in this population may partially be attributed to a high prevalence of LEA and risk of disordered eating (DE) and clinical eating disorders (EDs) among female and endurance athletes (Melin, Tornberg et al. 2015). Mechanical loading through weight-bearing exercises, such as high-impact loading activities and resistance training, provides an osteogenic stimulus and non-pharmacological approach to improving bone health (Hart, Nimphius et al. 2017, Beck, Daly et al. 2017). In a cross-sectional study of male distance runners, BMD was found to be significantly higher in runners who reported routine engagement in resistance training compared to runners who did not weight train and untrained controls (Duplanty, Levitt et al. 2018).

Despite the advantages of resistance training on bone health, recommending additional resistance training to athletes with LEA may further exacerbate energy deficiency if EI is not increased. Thus, physical activity interventions to counteract LEA must be designed to achieve maximal osteogenic responses with the minimal possible energy cost. Adding additional exercise without EI compensation may worsen the state of energy deficiency can cause further damage to the athlete's health. Even if an athlete does increase EI, adherence to additional routine resistance training is another potential issue. A survey of 667 competitive distance runners found only 60% of respondents engaged in routine resistance training, with middle-distance (800-3,000 m) runners reporting higher participation in strength and conditioning activities compared to long-distance (5k to half-marathon) runners (Blagrove, Brown et al. 2020). Middle-distance runners were 2.7 and 6.7 times more likely to engage in resistance training compared to long-distance and ultra-distance runners, respectively. An alternative to approach to resistance training that would

also apply mechanical loading to the bone is high-impact loading exercises like jumping and plyometric training. Brief jumping exercises have the potential to cause a significant osteogenic bone response with very little energy expenditure, given that a relatively low volume of 10-50 impacts/day at a frequency of 4-7 days/week is required to produce osteogenic effects in premenopausal women (Kishimoto, Lynch et al. 2012, Bailey and Brook-Wavell et al. 2010). The protocol in this proposed study will use jumping exercises at a volume of 5 sets of 10 jumps each day (50 jumps/day) with a 60 second rest between each set. Jumps will be at an intensity of 2x body weight and performed on 5 consecutive days. This approach has several benefits for runners with LEA, with the first being a relatively low energy cost of the jumping intervention which will prevent worsening the LEA state. The second benefit is the short time commitment required for high-impact loading exercises. Given the high running volume of most long-distance runners, engagement in additional cross-training such as resistance exercises may be a challenge. Thus, athletes may find it easier to adherence to the jumping exercises proposed in this study compared to other exercise interventions that require more time and effort. Based on the elevated risk of LEA and low BMD in female endurance runners, this population would benefit from this proposed study.

Findings from this proposed study will be of interest to sports dietitians and athletic trainers based on its potential to improve the clinical management of bone loss in female athletes with LEA through the use of brief, high-impact loading exercises. Future trials based on findings of the proposed study will likely explore the long-term efficacy of high-impact loading during prolonged LEA in active individuals across the spectrum of physical activity levels.

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 Biomarkers of Bone Remodeling in Blood including N-terminal propeptide of type 1 procollagen, undercarboxylated osteocalcin, sclerostin, and C-terminal telopeptide of type 1 collagen.

Change in Hormone and Metabolic Markers in Blood including parathyroid hormone, estrogen, insulin-like growth factor 1, hepcidin, insulin, cortisol, leptin, Nesfatin-1, and thyroid hormones
24-h Glucose

Secondary Endpoints

Change in Running Economy
Change in Body Weight
Change in ferritin, TIBC, iron, 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 loss (>5 lbs. during the 5-day experimental conditions)
Excessive change in absolute value for 24-hour glucose concentration
Excessive muscle soreness and/or pain
Fainting or light-headedness
Muscle or 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 randomized, cross-over intervention study that will evaluate the effect of brief, high-impact loading exercises on biomarkers of bone metabolism in energy-restricted, eumenorrheic female runners. Volunteers will complete two, 5-day experimental conditions in a randomized order separated by one menstrual cycle (approximately 3 weeks). Experimental conditions will include a dietary intervention of energy intake equal to 30 kcal/kgFFM/d using controlled diets and an exercise intervention of daily treadmill running with or without an additional 50 impact loading exercises.

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. Paired t-test will be used to detect differences in bone biomarkers and hormones within and between experimental conditions (Aim 1). Associations between undercarboxylated osteocalcin concentration and glucose metabolism (i.e., interstitial glucose concentration and serum insulin) will be analyzed using regression analysis (Aim 2). Data will be summarized as mean ± 1 standard deviation. 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, 233 Wallace Hall, and 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 study intervention will consist of controlled meals providing 30 kcal/kgFFM/d and exercise intervention (running with and without impact loading exercises) that will result in energy expenditure of 15 kcal/kgFFM. Menus will be designed by a registered dietitian (T.Sterringer) and provide 55% of total calories from carbohydrates, 20% from protein, and 25% from fat (example diet uploaded). A daily multi-vitamin will be provided during the nutrition intervention to ensure micronutrient needs are met during the acute energy restriction.

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 1 timepoint during the study (baseline). DXA scans will be performed by an ISCD Certified Bone Densitometry Technologist (T. Sterringer).

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:

A standard over the counter, multi-vitamin and mineral supplement (Nature Made) will be provided during the 5-days of controlled experimental diet to ensure micronutrient needs are met in the presence of energy restriction. Nature Made multivitamin supplements are verified by the United States Pharmacopeia (USP), a nonprofit organization that offers third-party verification of product quality and labeling accuracy.

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 at baseline. 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. Larson-Meyer has experience using CGM for research purposes as part of an ongoing research project (IRB #21-561).

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

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

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

N/A

8.0 Procedures Involved

8.1 Describe and explain the study design:

This study will employ a cross-over design in which 12 eumenorrheic women between the ages of 18 and 35 will complete two experimental conditions in a randomized order using a computer program with a random number generator. The cross-over study design

will help control for inter-subject variability in response to LEA (Guebels, Kam et al. 2014, Reed, De Souza et al. 2015).

As outlined in the table, eligible participants will undergo two, 5-day experimental conditions separated by one menstrual cycle (approximately 24 days) consisting of an energy-restricted diet and endurance training regimen. Experimental conditions will include a dietary intervention of energy intake equal to 30 kcal/kgFFM/d using controlled diets and an exercise intervention of daily treadmill running with one condition of run only (RUN) and one condition with running and 50 high-intensity, impact loading "jumping" exercises (RUN+IL) After providing informed consent, participants will be randomly assigned to the RUN or RUN+IL condition. Outcome data will include assessment of serum biomarkers of bone metabolism and circulating hormones important for bone metabolism. Compliance to the nutrition and exercise regimen will be evaluated at regular intervals and include collection of packaging and uneaten food at the end of each 5-day condition, measurements of body weight before and after each experimental phase to assess change, and physical activity data collected by accelerometer and digital technology provided by the research team (Garmin smart watch and My PT Hub app).

Dietary Intervention. During the two experimental conditions, participants will be provided controlled, weighed diets equal to 30 kcal/kgFFM/d. Energy intake will be manipulated individually based on the FFM of participants measured via DXA. Diets will be prepared by a registered dietitian (TS) in the research kitchen in Wallace Hall and standardized between conditions to include three meals and one snack. Menus will consist of similar whole foods and commercial products that provide approximately 55% of total calories from carbohydrates, 20% protein, and 25% fat. Diets will be modified based on participant allergies or preferences, within reason. Participants will be instructed to consume the meals and snack at approximately the same time each day to avoid within-day fluctuations in energy balance (Fahrenholtz, Sjödin et al. 2018). They will also be instructed not to consume any other foods or beverages other than water and non-calorie beverages (e.g., black coffee, unsweetened tea). A daily multi-vitamin will be provided to participants during the experimental conditions to provide adequate micronutrient intake during the energy-restricted state. The key investigators are both registered dietitians with experience assessing energy balance. The PI and has extensive experience conducting controlled feeding trials.

Exercise Sessions. Participants will undergo supervised exercise sessions consisting of treadmill running with and without high-impact exercises on 5 consecutive days in the NEM laboratory on two occasions separated by one menstrual cycle (approximately 24 days). On the first day of each experimental condition, exercise energy expenditure (EEE) will be measured using indirect calorimetry (ParvoMedics) during a controlled "titration" run to help determine running speed at 65% VO2max. A heart rate monitor will be used simultaneously to measure heart rate. Total EEE for the exercise session is approximately 15 kcal/kgFFM. From this, the running protocol (duration) needed to expend or "burn" a total of 15 kcal/kgFFM will then be determined and used throughout the study. In the RUN condition, participants will run on the treadmill run at that pace for the amount of time needed to expend 15 kcal/kgFFM each day of the five-day intervention. Running time on the treadmill will vary based on participant body weight, percent body fat, VO2max, and running efficiency. Treadmill duration will be

determined based on an expenditure of 15 kcal/kgFFM while running at an intensity of 65% VO2max. We estimate total running time for participants to fall within a range of approximately 50-65 minutes. For example, a runner with the following characteristics: body weight = 62kg, body fat % = 30%, VO2max = 55 ml/kg/min, would need to run for 58 minutes to meet an energy expenditure of 15 kcal/kgFFM. Runners with a higher running efficiency and VO2max will have slightly shorter running durations compared to runners who are less efficient. In the RUN+IL condition, participants will start by performing 5 sets of 10 high-impact loading jumping movements for a total of 50 impacts/session at intensities greater than 2x bodyweight with 60 seconds of rest between sets to stimulate an osteogenic response according to the guidelines for osteoporosis prevention recommended by the Exercise and Sport Science Australia (ESSA) (Beck, Daly et al. 2017). Intensity of the jumping exercises will be assessed using ground reaction force measured using dual force plates. Participant body weight will be obtained before each exercise session to ensure GRF of jumping exercises is at the desired intensity. The digital scale and monitor will be separate, and body weight will not be shared with the participant. Indirect calorimetry will also be used simultaneously on day one of the intervention (only) to assess the energy expended during the jumping exercises. Participants will then run on the treadmill at 65% VO2max until combined energy expenditure of impact loading and treadmill running reaches the target 15 kcal/kgFFM. Participants will also wear an accelerometer and smart watch devices during the experimental conditions to measure activity level. All activity tracking devices will be provided by the research team. Participants will be instructed to refrain from all physical activity outside of the supervised exercise sessions that are not related to activities of daily living (e.g., getting dressed, walking to the car).

Table 1. Overview of Data Collection

Overview of Data Collection								
			RUN and RUN+IL Experimental Conditions					
	Baseline	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Body Mass (weight)	x	x	x	x	x	x	x	x
Body Composition by DXA	x							
BMD (total body, dual femur, lumbar spine) by DXA	x							
Urine collection for hydration status	x							
Pregnancy test	x	x*						
Low Energy Availability in Females Questionnaire	x							
Vitamin D Questionnaire	x							
Blood via venipuncture								
CBC	x							
TSH	x							
Progesterone		x						
Vitamin D (total and free 25(OH)D)		x						

N-terminal propetide of type 1 procollagen		x						x
Undercarboxylated osteocalcin		x						x
Sclerostin		x						x
C-terminal telopeptide of type 1 collagen		x						x
Parathyroid hormone		x						x
Estradiol		x						x
Insulin-like growth factor-1		x						x
Hepcidin		x						x
Markers of iron status (Ferritin, iron, total iron-binding capacity [TIBC])		x						x
Insulin		x						x
Thyroid hormones (Free triiodothyronine (T3), free thyroxine and rT3)		x						x
Leptin		x						x
Cortisol		x						x
Nesfatin-1		x						x
Health outcomes								
Continuous glucose monitoring			x	x	x	x	x	
Compliance and Fitness Testing								
Habitual dietary intake (food records for 3 days)	x							
Habitual exercise tracking (smart watch for 3 days)	x							
Running Economy			x				x	
Aerobic Capacity (VO2max)	x							
Treadmill running at 65% VO2max			x	x	x	x	x	
Physical Activity tracking (smart watch)			x	x	x	x	x	
Mealtime Log			x	x	x	x	x	

Note: The 5-day intervention, RUN or RUN+IL, occurs on days 2-6

pregnancy test performed at only 2 timepoints: baseline and day 1 of the **second experimental condition after the 3-week washout period*

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 investigatior'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 will be used for phone screening conducted by a registered dietitian nutritionist.

Baseline Screening (approximately 1 hour)

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.

Low Energy Availability in Females Questionnaire (LEAF-Q): Subjects will be asked to complete the LEAF-Q, which will be used to screen for risk of existing LEA, medication use, menstrual function, and injury history. The LEAF-Q has been validated as a screening tool for LEA in female endurance athletes (Melin, Tornberg et al. 2014). Individuals who score as "high risk" for LEA will be provided referral information to a local sports dietitian.

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.

Bone Mineral Density: Total body, dual-femur, and lumbar spine BMD will be measured in all subjects via DXA scan. Scans will be conducted by T. Sterringer who is an ISCD Certified Bone Density Technologist.

Urine sample and pregnancy test: All participants will be required to provide a small cup of urine immediately before the DXA at baseline. 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 all participants.

Baseline Laboratory Testing Session (approximately 1 hour).

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.

Blood Draw: Blood will be drawn by venipuncture after a 12-hr fast (no caffeine) for the following: CBC, TSH, and progesterone. In total, blood will be draw five times during the study by venipuncture from a vein in the arm. These include baseline, and the days immediately before and after each experimental condition as explained below. Blood will be drawn with the participant seated quietly in a phlebotomy chair. Approximately 10 ml will be obtained during the baseline visit.

Aerobic capacity (i.e., 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 test will begin with a 5-minute warm-up at 0% grade and at a speed predetermined by the participant. Following the warm-up phase, the workload will be increased each minute by increasing treadmill speed or grade by 0.5 mph or 2.5%, respectively until the participants can no longer continue or volitional exhaustion is reached. The entire exercise testing protocol will last 12-20 minutes.

Habitual Diet and Exercise (3 days)

Habitual Diet and Exercise: Following laboratory testing, participants will be instructed on completing food records for 3 days (2 weekday, 1 weekend). Food records will be analyzed using Nutrition Data System for Research (NDSR; University of Minnesota), a dietary analysis software program. Participants will also wear a smart watch over this same time period that will be provided by the research team. 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 no GPS coordinates will be downloaded or recorded.

Vitamin D questionnaire: Participants will complete a questionnaire to assess vitamin D status over the previous month (DE Larson-Meyer). The vitamin D questionnaire includes a 52-item food frequency questionnaire, 6-item supplement section, and 7 questions related to sunlight exposure.

Intervention

After baseline screening and testing, eligible participants will be scheduled to complete two experimental conditions in a randomized order beginning on the second or third day of menstruation, depending on the participant's schedule and available appointments. Each experimental condition will require participants to come into the Nutrition and Exercise Metabolism (NEM) laboratory on 7 consecutive days. Table 1 outlines (section 8.1) provides an overview of data collection for each scheduled laboratory visit. On day 1, fasted blood draws will be collected in the morning after a 12-hour overnight fast and

body weight will be measured on a digital physician's scale. Participants will complete the LEA experimental trials on days 2-6. A follow-up blood sample will be collected on the morning of day 7 after an overnight fast and body weight will be measured on a digital physician's scale.

Pregnancy Test: a pregnancy test will be performed after the 3-week washout period on day 1 of the second experimental condition.

Blood Draw: Blood (approximately 20-25 ml) will be drawn the days before and after each LEA experimental trial by venipuncture after a 12-hr fast (no caffeine) for the following: Progesterone, total and free 25(OH)D, N-terminal propetide of type 1 procollagen, undercarboxylated osteocalcin, sclerostin, C-terminal telopeptide of type 1 collagen, parathyroid hormone, estradiol, insulin-like growth factor, hepcidin, ferritin, TIBC, iron, cortisol, leptin, nesfatin-1, insulin, free T3, free T4, rT3. In total, blood will be draw five times during the study by venipuncture from a vein in the arm with a total of 110 ml obtained for the duration of the study. Blood will be drawn with the participant seated quietly in a phlebotomy chair.

Dietary Intervention: During the two experimental conditions, participants will be provided controlled, weighed diets of 30 kcal/kgFFM/d. Total energy of diets will be individualized based on the participant's FFM measured via DXA. Diets will be prepared by a registered dietitian (T. Sterringer) in the research kitchen in Wallace Hall and standardized between conditions to include three meals and one snack. Menus will consist of the same whole foods and commercial products that provide diets consisting of approximately 55% carbohydrates, 20% protein, and 25% fat. For example, a woman weighing 54.5 kg (120 lbs.) with a body fat percentage of 22% (FFM = 42.5 kg) would be provided with a diet containing 1275 kcal (55% total calories = 175g carbohydrates, 20% = 64g protein, 25% = 35g fat) based on 30 kcal/kgFFM (example menu uploaded). Participants will be instructed to consume the meals and snack at approximately the same time each day to avoid within-day fluctuations in energy balance (Fahrenholtz, Sjödin et al. 2018). They will also be instructed not to consume any other foods or beverages other than water and non-calorie beverages (e.g., black coffee, unsweetened tea). A daily multi-vitamin will be provided to participants during the experimental conditions to ensure adequate micronutrient intake during the energy-restricted state. Participants will be asked to log the times they consume each meal and snack in a mealtime log for the 5-day experimental phases. The primary investigator (E. Larson-Meyer) is a registered dietitian and has extensive experience with assessment of energy balance with controlled feeding trials.

Exercise Sessions: Participants will undergo supervised exercise sessions consisting of treadmill running with and without high-impact exercises on 5 consecutive days in the NEM laboratory on two occasions separated by one menstrual cycle (approximately 24 days). On the first day of each experimental condition, exercise energy expenditure (EEE) during the sessions will be measured using indirect calorimetry (ParvoMedics) and heart rate to ensure total EEE is approximately 15 kcal/kgFFM. In the RUN condition, participants will complete a 5 min warm-up (at 5.5, 6 or 6.5 mph) and then run on a treadmill at 65% VO2max for the duration determined at baseline (see

protocol in section 8.1) to elicit an EEE of 15 kcal/kgFFM. In the RUN+IL condition, participants will perform impact loading movements (jumping) and a running protocol. Participants will come to the lab in the morning to perform 5 sets of 10 impact loading movements for a total of 50 impacts/session at intensities greater than 2x bodyweight. Each jumping set will be separated by 60 seconds of rest in alignment with guidelines for osteoporosis prevention recommended by the Exercise and Sport Science Australia (ESSA) (Beck, Daly et al. 2017). Ground reaction force of impacts will be measured using dual force plates. Approximately 4-6 hours later, participants will return to the lab to complete a 5-min run and warm-up on the treadmill at 65% VO2max until a combined energy expenditure of the morning impact loading exercise and afternoon/evening treadmill running reaches 15 kcal/kgFFM. A recovery period of at least 4 hours is recommended between impact loading sessions for optimal osteogenic bone response (Hart, Nimphius et al. 2017). Participants will wear accelerometers and smart watch devices during the experimental conditions to measure activity level. Participants will be instructed to refrain from all physical activity outside of the supervised exercise sessions that are not related to activities of daily living (e.g., getting dressed, walking to the car).

Body Weight: Body weight will be obtained before each supervised exercise session begins using a digital physician's scale to monitor significant changes in body weight during the intervention. Participant body weight will also be needed for monitoring the impact of the jumping exercises using ground reaction force in the RUN+IL condition. The scale and monitor will be separate and daily body weight will not be shared with participant.

Ground Reaction Force (GRF): Ground reaction force of jumping exercises will be performed using dual force plates. Participants will perform 5 sets of 10 vertical jumps with 60 seconds of rest between each set. Jumping exercises will be tailored until intensity reaches a threshold of at least 2x body weight of participant. These jumping exercises will be used in the RUN+IL condition.

Running Economy: Running economy will be assessed on the first and last day of each experimental condition at the start of each supervised treadmill run. Heart rate will be measured during the test by a heart rate strap and sensor (Polar). The test will begin with participant running for 4-minutes at three moderately easy speeds of 5.5, 6 6, and 6.5 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). This test will last 12 minutes. Following cumulation of the standard economy test, the workload will be increased to the 65% VO2max. Oxygen consumption will be measured for the first 4 to 10 minutes of the steady state run.

Continuous Glucose Monitor (CGM): Interstitial glucose concentration will be measured during the two, 5-day experimental conditions using a CGM device which 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 5-day experimental conditions. CGM will be placed on the day before each LEA condition and removed on the day after each condition.

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.

Energy Restriction: Potential risks associated with energy restriction include loss of lean body mass (LBM), suboptimal macronutrient and micronutrient intake, hypoglycemia, and mood disturbances. These risks will be minimized by providing a daily multivitamin to participants for the duration of the 5-days of energy restriction. Menus will also be designed to provide 20% of total calories from protein to help preserve LBM. To reduce risk of hypoglycemia, diets will provide 55% of total calories from carbohydrates and participants will be encouraged to consume the provide snack containing 30g carbohydrate approximately 30 minutes before the exercise sessions. Additionally, menus will be designed to prioritize foods with fiber and low energy density that promote fullness and satiety (e.g., leafy greens, fresh non-starchy vegetables, whole grains and other high-fiber foods) to reduce physical and psychological burden of energy restriction. Participants with existing menstrual disturbances (determined by progesterone and self-report), LEA (determined by LEAF-Q score), and/or low BMD (determined by DXA) will be excluded from the study to prevent worsening existing conditions.

High-Intensity Exercise: Potential risks associated with endurance running and high-impact loading exercises include musculoskeletal injuries, changes in blood pressure, gastrointestinal discomfort, fainting, and dizziness. These risks will be minimized by

having participants assessed prior to each exercise session by a trained research member to evaluate readiness for physical activity. Additionally, participants will complete all exercise sessions in the lab under the supervision of a certified strength and conditioning specialist with CPR certification (T. Sterringer).

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) 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 five times during the study (baseline, RUN day 1, RUN day 7, RUN+IL day 1, RUN+IL day 7) with a total of 110 ml of blood collected over the course of the 5-6 weeks.

DXA scan: Participants will be exposed to a very low dose of ionizing radiation as part of the DXA scan at baseline only. 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 and basic demographic (age) data will be recorded on data sheets and manually entered into a database (excel format) on a secure computer. Select data (DXA results, VO2max, ground reaction force) may be transferred electronically directly from the DXA, metabolic cart, or force plates into excel spread sheets, if possible. Blood 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.

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 based on cost savings. 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 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 and authorized study personnel (doctoral student in charge of the study, T. Sterringer). 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, BMD, fitness, and pertinent blood markers. 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 6 to 10 weeks, which will include a Zoom or telephone call for screening, a screening visit in the laboratory, a baseline visit, three days of habitual diet and exercise tracking, two experimental conditions, and a washout period of 1 menstrual cycle (approximately 3 weeks). 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 6 months based on enrollment of 1-2 participants per week. 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 will be used for this purpose conducted by a research team member who is also a registered dietitian nutritionist (RDN). The Low Energy Availability in Females Questionnaire will be used to screen for risk of existing LEA, medication use, menstrual function, and injury history.

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):

Eligible participants will be well-trained, eumenorrheic female runners between the ages of 18-35 years that are weight-stable with a BMI between 18.5-30 kg/m^2. Participants with LEAF-Q scores ≥8, existing menstrual disturbances measured by progesterone and self-report, or low BMD (z-score <-2) will be excluded from the study to prevent worsening existing conditions and recommended to follow up with their primary care physician and/or a registered dietitian nutritionist. During the phone screening, participants will be asked if they have ever been diagnosed with an eating disorder and the circumstances surrounding the diagnosis. If the participant has just recently recovered (within the last 12 months) or is still in recovery, she will be excluded from the study. The LEAF-Q was not developed to assess disordered eating behavior, however, a study in ultra-marathon female runners found a significant association between high LEAF-Q scores and disordered eating (Folscher, Grant et al. 2015). Athletes who score 8 or higher on the LEAF-Q will be excluded from the study and referred to a local sports dietitian. Participants using contraceptives (oral contraceptives, injections, IUD, etc.) will be excluded from this study. Contraceptive use may mask menstrual irregularities and oral contraceptives may influence glucose metabolism (Lopez, Schultz 2007), a secondary outcome of this study. Adequate training status for the study protocol will be assessed based on running volume, frequency, and VO2max. Eligible participants must be able to run on 5 days/week for at least 60 minutes to meet the training requirements of the study protocol. Additional exclusion criteria include history of fracture in previous 6 months, medication use that could potentially affect bone metabolism (e.g., corticosteroids, anticonvulsants, heparin, gonadotropin-releasing hormone agonists), pregnancy, lactation, abnormal TSH, and routine engagement in mechanical loading exercises. Participants must be willing to consume the diets provided, however, diets will be modified based on patient allergies or preferences, within reason. Participants will be excluded if they have dietary restrictions or preferences that would prevent them from consuming meals that fit within the experimental conditions. For example, we cannot modify diets to meet low-carbohydrate preferences given the experimental diets must contain 55% of total calories from carbohydrates. Similarly, participants who are unable to consume high-fiber diets will be excluded.

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 because intentional energy restriction is not appropriate during pregnancy. A pregnancy test will be performed at baseline prior to the DXA scan and after the 3-week washout period on Day 1 of the second experimental condition.

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):

A power analysis determined that 12 women will be required to detect a clinically significant change of 10µg/L in PINP with 80% power at $P < 0.05$ based on results from a similar study of LEA during dietary restriction (Papageorgiou, Martin et al. 2018). We aim to enroll 20 participants to account for possible dropout.

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 50-60 participants to recruit and complete the required 12 participants.

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

All enrolled participants will undergo the two experimental procedures.

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, and recreational centers, etc.) at colleges and universities in the New River Valley (including Virginia Tech), local running stores, running groups in New River Valley, 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. This will include recruiting members of local running groups and residents of New River Valley. We welcome diverse participants from all racial, ethnic, educational, financial, and social backgrounds. Given the nature of this laboratory-based intervention, subject recruitment will be limited to individuals residing in the New River Valley and surrounding areas. Increased recruiting efforts will be targeted at Radford City County given the increased diversity of this county compared to the other 4 counties in New River Valley.

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 "Volunteers needed for study on bone health of long-distance runners". Participants will be compensated $25 for completing the baseline visit and first experimental condition (7 days). Participants will be compensated an additional $75 for completing the second experimental condition. This is a total of $100 compensation for completing all testing visits and experimental conditions. Participants that do not complete the second condition will receive information about body composition, BMD, and aerobic fitness (VO2max, running economy), in addition to the $25 compensation. Given the cross-over design of this study, data cannot be used for participants that do not complete both conditions. The experimental conditions will be separated by 3 weeks of their normal diet and exercise routine and not require any lab visits or intervention. The total time commitment for this study will be 19 days. Payment in the form of cash will be scheduled after each individual participant completes each experimental condition.

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 exercise sessions, are not consuming the provided diets, 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 another member of the study staff 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, BMD). 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)*

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 the exam (including total body, femur, and lumbar spine) 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.

Running Economy, Exercise Capacity (VO2max), and Treadmill Runs: 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 participants who are involved in running and other exercise activities. 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. Risks associated with treadmill running will be minimized by recruiting only participants who have current experience with long-distance running at a high volume (at least 30 miles per week) and frequency (at least 5 days per week) to meet the exercise demands of the protocol.

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: Experimental diets will induce a state of low energy availability through dietary energy restriction. Potential risks associated with energy restriction include loss of lean body mass (LBM), suboptimal macronutrient and micronutrient intake, hypoglycemia, feelings of weakness and mood disturbances. These risks will be minimized by providing a daily multivitamin to participants for the duration of the 5-days of energy restriction. Menus will also be designed to provide 20% of total calories from protein to help preserve LBM. To minimize risk of hypoglycemia, diets will provide 55% of total calories from carbohydrates and a snack will be provided to participants containing approximately 30g carbohydrate to be consumed prior to the treadmill run. Additionally, menus will be designed to prioritize foods with low energy density that promote satiety (e.g., high fiber foods) to reduce physical and psychological burden of energy restriction. Participants with existing menstrual disturbances, LEA, and/or low BMD will be excluded from the study to prevent worsening existing conditions. Given the short-duration of this study and the washout period of approximately 24 days between LEA conditions, it is unlikely the acute energy restriction will result in any long-term consequences.

High-Impact Loading Exercises (Jumping): Potential risks associated with high-impact loading exercises include musculoskeletal injuries and excessive bone strain. These risks will be minimized by excluding participants with recent history of bone stress injuries or low BMD (z-score <-2.0). Additionally, the exercise sessions will be supervised by an NSCA-certified strength and conditioning specialist (T. Sterringer) with an emphasis on proper form and safety techniques. 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.)

Weight loss will be expected during the two, 5-day intervention periods because the participants will be in a state of low EA (15 kcal/kgFFM/d). Previous studies (Ihle, Loucks 2004, and Loucks, Thuma 2003) observed an average weight reduction of 2.0-2.2 kg and 1.1-1.2 kg in young women (average age 21 years) in response to EA treatments of 10 and 20 kcal/kgFFM/d, respectively, over a 5-day period. This study will use a safety endpoint of excessive weight loss defined as 5-lbs (approximately 2.3 kg) over the 5-day energy-restricted intervention phases.

Other safety measures addressed 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 soreness in legs by participating in our high-volume running regimen on 5 consecutive days and during the 5-day intervention with high-impact jumping. It is also possible an unknown allergy to foods contained in the controlled diets could be identified during the study. These events, however, are not likely. To minimize the potential, we will only recruit participants with current long-distance running experience at a high volume (at least 30 miles per week) and frequency (at least 5 days per week) to meet the exercise demands of the protocol.

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

Dietary restriction is a risk to the development of the fetus should the subject become pregnant during the study intervention. Pregnancy tests will be performed at baseline testing (specifically before the DXA) and after the 3-week washout period on Day 1 of the second experimental condition, to ensure the participant is not pregnant. Progesterone will be measured to ensure menstrual status. Participation in the study would end if it were determined the participant has 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 bone mineral density, body composition and running fitness (VO2max and running economy). They will also learn how to properly perform high-impact loading 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:

Dr. Larson-Meyer has extensive experience performing data collection the procedures (or similar procedures) as does Dr. Elaina Marinik. Dr. Marinik and Trisha Sterringer are ISCD Certified Bone Densitometry Technologists. Additionally, T. Sterringer is a NSCA Certified Strength and Conditioning Specialist and has experience or been trained on performing the procedures detailed in this protocol. The research team will ensure all study personnel 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 exercise economy, VO2max, and exercise energy expenditure 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) 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. 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 and the Low Energy Availability in Females Questionnaire. 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):

Study participants will be informed during the baseline screening and exercise sessions that they can discontinue the study and/or exercise session at any time without penalty. 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, fitness testing and the supervised exercise 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, or unexpected events/issues during the 5 days of consecutive running and 5 days of consecutive running with impact loading exercises. 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 body weight or interstitial glucose concentration that will measured throughout the 5 days of energy restriction in the experimental conditions.

The graduate student in charge of the project (T. Sterringer) will consult with Drs. Larson-Meyer and Marinik and be responsible for assembling the data, producing reports, and 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 authorized study personnel

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. 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:

N/A

23.0 Economic Burden to Subjects

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

The participant will be responsible for costs that may include purchase of athletic clothes or shoes to participate in the running and impact loading exercise 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 (phone script and screening form uploaded). 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 (email template uploaded).

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, 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 doctoral student in charge of the study (T. Sterringer). 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, when they need to notify the team about the start of menstruation, how often they will be asked to consume the meals and snacks provided by the research team, what foods and beverages they can consume outside of the ones provided by the research team, what physical activities they can do outside of the ones conducted in the NEM lab, and how often they would need to come to the lab to run.

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.*

Only English-speaking participants will 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 current training status, running frequency and volume, dietary restrictions, medication use, injury history, 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 oversees four doctoral students. 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 will dedicate time to this study and ensure the doctoral student is adequately trained and performs all aspects of the study according to protocol and procedures. The doctoral student, Trisha Sterringer, will be in charge of participant recruitment and enrollment, protocol execution, data collection and the day-to-day aspects of the study. T. Sterringer is a registered dietitian (RD), NSCA certified strength and conditioning specialist, and ISCD Certified Bone Densitometry Technologist. She has previous experience conducting energy availability studies in competitive athletes at Case Western Reserve University involving indirect calorimetry, and assessments of dietary intake, exercise energy expenditure, and body composition. Trisha Sterringer will be the doctoral student in charge of the study as part of her PhD dissertation, however, additional undergraduate research assistants may be added to the study in the future. Dr. Elaina Marinik has extensive experience in study management and coordination at Virginia Tech and will be involved in the study to assist as needed. There are several key laboratories in the HNFE Department that will be utilized for this proposed study as described below.

The Nutrition and Exercise Metabolism (NEM) Laboratory is directed by DE Larson-Meyer and located on the Virginia Tech Corporate Research Center campus. Major equipment items in the NEM Laboratory include Parvomedics TrueOne 2400 metabolic cart, private room with a table for completing questionnaires, Woodway treadmill, and refrigerator and freezer storage. Free parking is available on-site and restrooms, showers, and changing facilities are also available.

The Laboratory for Eating Behaviors and Weight Management (Director: Brenda 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 anthropometrics, and a file storage area. Also located in Wallace Hall 233 is a Lunar iDXA (GE Healthcare) and space for sample processing.

The Human Integrative Physiology Laboratory is located on the Corporate Research Campus at the Garvin Innovation Center. The major equipment items in this laboratory include Lunar Prodigy DXA (GE Healthcare) and space for sample processing (i.e., wet lab areas). Additional research space is also available in Wallace Hall for sample processing (i.e., wet lab areas) and storage (-80 freezers).

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