Characterizing the Use of Continuous Glucose Monitoring During Experimentally-Induced Short-Term Low Energy Availability in Female Endurance Runners

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Abstract: Female endurance runners are at high risk for low energy availability (LEA). Preliminary evidence suggests that fasting blood glucose (BG) concentration decreases in response to short-term energy deficiency (low energy availability). BG monitoring tools, such as continuous glucose monitors (CGM), could be used as an early detection device for LEA risk in athletes. PURPOSE: Characterize changes in BG over the course of five days in experimentally-induced LEA in female runners. METHODS: Recreational runners completed a 5-day experimental condition of LEA (15 kcal/kg FFM/d) achieved by a combination of dietary restriction and treadmill running at 65% VO_{2max}. BG concentration was monitored every 15 min across the five experimental days using a CGM (Freestyle Libre Pro, Abbott), which assesses BG from interstitial glucose concentration. Glucose data from the 5-day condition were analyzed to obtain average 24- h BG, fasting BG, BG during sleep, "time in target" BG range (TIT), "time above target" range (TAT), "time below target" range (TBT), as well as glycemic variability using MAGE (mean of amplitude of glucose excursions) and MODD (mean of daily differences). 70 to 120 mg/dL was set as the target range. Data was analyzed using repeated measures analysis of variance; post hoc comparisons were performed using paired t-tests. RESULTS: Contrary to our hypothesis, our results suggest that five days of experimentally-induced LEA in female runners progressively increased 24-h BG and TIT while simultaneously altering patterns of fasting and sleeping BG. Average glucose concentration and TIT significantly increased from day one to day five (P=0.024 and P=0.03, respectively). Fasting and sleeping BG followed the same trends and significantly decreased from day one to day three (P=0.04 and P=0.002, respectively), followed by an increase by day five that was similar to day one. There was not a significant time-effect for MAGE, MODD, TBT, TBT, and glycemic variability (P>0.05). These changes are

likely due to alterations in glucose production versus utilization that are driven by decreased insulin and/or increased BG counterregulatory hormones. This study is the first to characterize glycemia during short-term experimentally-induced LEA in female endurance runners using a CGM; emphasizing the potential ability of CGMs to gain insight on BG patterns during conditions of LEA.

Characterizing the Use of Continuous Glucose Monitoring During Experimentally-Induced Short-Term Low Energy Availability in Female Endurance Runners

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General Audience Abstract: Female long-distance runners are at high risk for a lack of proper fueling due to inadequate energy intake compared to the volume they exercise (e.g. low energy availability). Previous studies have found that fasting blood sugar (BS) concentration decreases in response to a short-term bout of low energy availability. BS monitoring tools, such as continuous glucose monitors (CGM), could be used as a tool to detect this risk in athletes. PURPOSE: Characterize changes in BS over the course of five days in experimentally induced low energy availability in female runners. METHODS: Recreational runners completed a 5-d experimental condition of low energy (15 kcal/kg FFM/d) achieved by a combination of dietary restriction and daily treadmill running at 65% of their maximum performance capacity (e.g. VO_{2max}) BS concentration was monitored every 15 min across the five experimental days using a CGM (Freestyle Libre Pro, Abbott). Glucose data from the 5-d condition were analyzed to obtain average 24- h BS, average BS during sleep, fasting BS, "time in target" (TIT), "time above target" (TAT), "time below target" (TBT), the glycemic variability (GV) as percent coefficient of variation, and swings in BS levels (e.g. glycemic variability) using calculations for mean of amplitude of glucose excursions (MAGE), mean of daily differences (MODD). Data was analyzed using repeated measures analysis of variance; post hoc comparisons were performed using paired t-tests. Data was summarized as a mean ± standard deviation. The significance level was set a priori at $P<0.05$. All statistical analysis was conducted using IBM® SPSS® Statistical software (Version 28.0.2.2, IBM Corporation, NY, USA). RESULTS: Our results suggest that five days of experimentally-induced LEA in female runners progressively increases TIT and 24- h BS while simultaneously altering patterns of fasting and sleeping BS. Average glucose concentration and TIT significantly increased from day one to day five ($P=0.025$ and $P=0.03$, respectively). Fasting and sleeping BS significantly decreased from day one to day three ($P=0.024$ and

P=0.002, respectively) and had the same trends. The concentrations of both fell from day one to day three, followed by an increase by day five that were like those of day one. Additionally, there was not a significant time-effect for MAGE, MODD, TBT, TBT, and glycemic variability all had an insignificant time-effect (P>0.05). These changes are likely due to alterations in glucose production compared to glucose use that are driven by changes in the hormones that regulate blood sugar. This study is the first to characterize BS changes during short-term experimentally-induced LEA in female endurance runners using a CGM; emphasizing the potential usefulness of CGMs to gain further insight on BS patterns during conditions of LEA.

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Chapter 1. Introduction

Carbohydrate (CHO) has long been known as the most vital fuel source for athletes' exercise performance; Adequate CHO optimizes training, delays fatigue, and promotes recovery [1]. Low CHO availability can be a detriment to the performance of prolonged submaximal or intermittent exercise (i.e., swimming, cycling, running)[2]. Metabolically, this is related to insufficient liver and muscle glycogen concentrations coexistent with increased exercise energy expenditure, the former resulting in a fall of blood glucose (BG)[3]. To fully understand the importance of CHO for athletes, it is also important to understand blood glucose (BG) homeostasis to facilitate optimal physiological conditions for performance. BG homeostasis is central to the success of an exercise bout. Studies of such findings date back to 1924 when Levine and colleagues[4] found that athletes who experienced lower BG (hypoglycemia) had a decrease in performance (i.e. fatigue, speed) and overall well-being after a marathon race. To combat hypoglycemia, athletes were recommended to consume a higher CHO diet before the same marathon the following year or consume candy during the event, which resulted in a maintenance of normal BG concentration and improvements in physical condition during competition. Even with this insight, proper fueling continues to be an issue across many sports on an individual basis[5,6] and may be particularly a concern in athletes with LEA.

To further understand the individual CHO needs of an athlete, BG monitoring is a feasible option to combat under-fueling in sports. Continuous glucose monitors (CGM) are an emerging method of measuring interstitial fluid glucose (ISF) at rest and during exercise in athletes. CGMs were first brought to the market to help with clinical management of insulin-dependent and non-insulin-dependent diabetes[7]. However, manufacturers of CGM devices have either identified or created a wider interest among athletes to maintain optimal concentration during training and competition[7].

Athletes that participate in prolonged submaximal or intermittent exercise are at risk for low energy availability (LEA)[5,8,9]. Generally, women have a higher prevalence of LEA than men[9]. In this condition, insufficient energy

is consumed to support resting metabolic rate and non-exercise activity thermogenesis (the energy expended for everything we do that is not sleeping, eating, or exercising). Consequences of LEA include compromised physiological processes such as decreased bone health, gastrointestinal and cardiovascular dysfunction, disrupted glucose homeostasis, and irregular menstruation; all of which can contribute to impaired sports performance or injury risk[5,10]. Previous studies measuring BG during conditions of LEA have found that there is a decreases in BG in response to severe energy restriction (e.g. 15 kcal/kg FFM/day)[5,8,9], and that there is also a noticeable link between LEA and overtraining with CHO intake as a confounding factor[11,12].

Considering the higher prevalence of LEA in women than men, along with the preliminary evidence which correlates BG and LEA, there is an opportunity for CGMs to be utilized as a tool to detect LEA in female athletes. Using CGM data to prevent a prolonged state of LEA could be an advantage to health and performance outcomes. Little data is available on the characteristics of BG fluctuations in female athletes with LEA. Therefore, CGMs may serve as a useful tool to further investigate the relationship between BG and LEA in this population.

Chapter 2. Literature Review

Overview of BG Regulation:

Most cells in the body utilize glucose as the main source of energy, including those of the brain, liver, adipose tissue, and cardiac and skeletal muscle[12]. BG utilization for cellular energy generation is a tightly regulated process that differs at rest and during exercise. The breakdown of CHO into glucose by the gut upon consumption is a primary source of energy. CHO is then stored in the liver and muscle as glycogen (which is a multi-branched polysaccharide of glucose); glycogen is important for maintaining glucose homeostasis.

Liver glycogen is commonly referred to as the "glucose-regulator" because it serves as a readily available supply and helps maintain blood glucose homeostasis[13,14]. On average, the adult liver stores approximately 80 to 110 grams of glycogen in the postabsorptive state[12]. From these stores, glycogen can be broken down to glucose (via glycogenolysis) and released into circulation. Gluconeogenesis also occurs in the liver (as liver glycogen stores approach depletion) and is the process where glucose is synthesized from mostly non-CHO carbon sources including glycerol, and carbon skeletons of amino acids[12].

In contrast, muscle glycogen is a readily available energy source for working muscle. At rest, skeletal muscle has a total glycogen content of approximately 300 to 400 grams of carbohydrates[13]. Glycogen cannot serve as a direct source of blood sugar because it lacks the glucose-6-phosphate enzyme, which enables the release of glucose into the blood[14]. As an aside, phosphorylation of glucose by hexokinase is insulin dependent, and upon uptake into tissues "traps" glucose and prevents its transport across the membrane[13]. During exercise, however, carbons from muscle glycogen can exit muscle as lactate (or pyruvate). Lactate may serve as a source of blood sugar through the breakdown of glucose under anaerobic conditions or when cellular energy demands are high. Lactate is then converted to glucose and released into the blood to help maintain glucose homeostasis (i.e., the Cori or glucose-lactate cycle)[13,15].

The brain depends on glucose as its main source of energy. The adult brain utilizes approximately 20% of glucose-derived energy (~5.6 mg glucose per 100 g human brain tissue per minute)[13,16]. After an overnight fast, liver glycogen utilization rate in the brain can be reduced to concentrations as low as 0.01 g per minute in resting conditions[13].

In healthy individuals without diabetes, BG is tightly regulated by hormones that include insulin, glucagon, epinephrine, cortisol, and growth hormone. Following a meal or snack, BG concentrations rise immediately after eating and typically return to pre-meal concentration by two h after eating. A normal range of BG is called euglycemia and ranges from 70 to 120 mg/dL (5 to 7 mM). In the fasting state, the normal range is considered 70 to 100 mg/dL[16]. A drop below 54 mg/dL (3mM) is considered hypoglycemia. Typical signs and symptoms associated with hypoglycemia include hunger, trembling or shaking and sweating. With chronically low blood sugar (typically associated with type 1 diabetes), adverse effects of weakness, blurred vision, skating, sweating, confusion, or numbness may occur[16]. In more extreme cases, seizures, coma, and very rarely death are possible. Conversely, BG concentrations above 126 mg/dL (7 mM) are considered hyperglycemia. Consistently high BG (typically associated with type 2 diabetes) increases the risk of damage to the eyes, nerves, kidneys, and blood vessels[16].

In the fed state, circulating glucose mostly comes from CHO consumed in the diet via digestion and subsequent transport of glucose from the small intestine to the liver and into circulation. In contrast, in the fasting state, glucose is released from the liver via glycogenolysis, and gluconeogenesis as previously mentioned.

<u>Hormones and BG:</u>

Hormones play a crucial role in BG regulation[17]. The major hormones involved in BG regulation are described in T*able 2.1*. In resting conditions, insulin is the most important glucoregulatory hormone. Insulin is released by pancreatic beta cells and stimulates the uptake of glucose into various tissues. After a meal, plasma insulin

concentrations increase, and as a result, glucose uptake by muscles, liver, and other tissues is increased. Along with this, insulin also promotes the storage of glucose as glycogen in the liver and the skeletal muscle[17]. In contrast, epinephrine (also known as adrenaline) acts directly on the liver to promote glucose production via glycogenolysis and suppresses insulin secretion[16]. Glucagon is another crucial glucoregulatory hormone that plays a role in maintaining BG concentrations. Glucagon is counteractive to insulin by stimulating hepatic glucose output and thereby increasing blood glucose concentration. It is released in response to a drop in blood glucose concentration and is important in maintaining blood glucose concentration between meals[13]. Specifically, glucagon signals the liver to break down stored glycogen into glucose (i.e., glycogenolysis) and helps form new glucose units via gluconeogenesis.

Other hormones that play a role in glucose homeostasis include somatostatin, amylin, cortisol, and growth hormones (GH). Somatostatin is released from the pancreas and works to prevent the release of the pancreatic hormones (i.e., insulin, glucagon, gastrin, and pancreatic enzymes that aid in digestion)[14] . This lowers blood glucose concentrations as a secondary effect of the inhibition of glucagon secretion. Amylin regulates postprandial insulin by decreasing glucagon concentrations. This decreases hepatic glucose output and increases satiety, which then acts to slow the rate of gastric emptying from the stomach and thereby the postprandial rise in blood glucose[17]. On the other hand, cortisol is a steroid hormone that promotes resistance to insulin action in skeletal muscle and adipocytes cells and enhances gluconeogenesis and glycogenolysis, thereby increasing BG concentration[14]. Similarly, growth hormone counterbalances insulin in skeletal muscle and adipocytes by inducing gluconeogenesis and glycogenolysis and promoting insulin resistance[14,17].

Table 2.1: Major Hormones involved in BG Regulation

Hormone	Gland	Stimulus	Action
Insulin	Pancreas	Increase BG	Helps transport BG into cells; decrease BG concentrations

Glucagon	Pancreas	Decrease BG; exercise stress	Promotes gluconeogenesis in the liver; helps increase BG concentrations
Epinephrine	Adrenal	Exercise stress; decrease in BG	Promotes glycogen breakdown and glucose release from the liver; helps increase BG concentrations
Cortisol	Adrenal	Exercise stress: decrease in BG	Promotes breakdown of protein and resultant gluconeogenesis; helps increase BG concentrations

Source modified from Textbook: Nutrition for Health, Fitness, and Sport. (n.d.).

BG During Exercise:

Maintenance of blood glucose becomes more complex during exercise due to the increased demands of glucose utilization as a fuel source. This can be explained by a phenomenon known as the cross-over effect, which is when the primary form of energy during exercise transitions from fat to CHO (~ 60 to 75% VO_{2max})[18]. The rate at which muscle glycogen is oxidized depends mostly on exercise duration and intensity where skeletal muscle is responsible for 90% of glucose uptake. During exercise, both hyper and hypoglycemia (i.e., a rise and fall in blood glucose above and below the normal physiological range, respectively) are possible and dependent on the intensity and duration of the exercise, previous or recent fueling, training state of exerciser and external conditions (external temperature, altitude, etc.)[19].

During moderate-intensity exercise (~ 60% VO_{2max}) in persons without diabetes, increased glucose uptake by skeletal muscle is balanced by an equal rise in liver glucose production and BG concentration[13]. This resembles glucoregulation in the basal state (e.g., glucose released from the liver is controlled by glucagon and insulin). There is a decrease in insulin concentration with exercise initiation, which stimulates glycogenolysis, thus increasing glucose output. During prolonged moderate-intensity exercise (i.e. greater than two h), epinephrine plays a role in increasing glucose output (i.e. gluconeogenesis)[20]. This is typically due to a lack of adequate

fueling and thus, not enough liver glycogen left in storage. Gluconeogenesis then occurs and is typically accompanied by a decline in plasma insulin concentration. The longer the exercise, the greater the relative contribution of gluconeogenesis to liver glucose production and output.

Intense exercise (>80% VO_{2max}) is like a stress response. Muscle glycogen breaks down rapidly and becomes nearly depleted in a short period. BG is no longer closely regulated; the liver releases glucose at a higher rate than it is taken up by muscle and there is a disproportionate increase in plasma epinephrine. The result is an elevation of BG concentrations during intense exercise that further increases immediately after exercise and can persist for up to one h. As a result, plasma insulin concentration rises post-exercise to restore the elevated glucose concentrations[20].

Even in an acute bout of exercise, insulin sensitivity and glucose effectiveness increase (independent of insulin), resulting in late hypoglycemia[18]. Much of this phenomenon is thought to be due to an increase in GLUT4, an insulin-dependent transporter of glucose which translocate intracellularly to the plasma membrane upon muscle contraction. GLUT4 in muscle and adipose tissue results in the predominant use of CHO as a fuel source[18].

<u>BG in Athletes:</u>

The regulation of blood glucose during exercise is also known to be influenced by training status. Trained athletes are typically more sensitive to insulin resistance. This is because long term training both reduces hepatic glycogenolysis and gluconeogenesis production and increases the responsiveness of the adrenal medulla to exercise, thus increasing secretory capacity. This involves the increase in secretion of GH, beta endorphin, and epinephrine, while decreasing glucagon and insulin. LeBlanc and colleagues[21] investigated this phenomenon by analyzing the response to insulin injections in athletes compared to untrained subjects. In trained athletes, there was a greater fall in plasma glucose concentration for the same amount of insulin due to high insulin sensitivity and a higher raise in GH and epinephrine. Another training adaptation includes a

reduction in muscle glucose transport from an increase in muscle mitochondrial respiratory capacity. A combination of these mechanisms lowers an athlete's reliance on CHO and increases utilization of lipids[19].

The BG adaptations that come with regular training, however, may be dampened under conditions of both overtraining and overreaching. Overtraining is when training volume or intensity of exercise increases to the point of performance decrements whereas over-reaching is defined as a less severe condition where recovery can take a few days[19]. With overtraining, responses of growth hormones and cortisol to both insulin-induced hypoglycemia and exercise are impaired (e.g., counter-regulation is less efficient). Hypoglycemia is a common symptom of overtraining due to the alterations in binding proteins for insulin-like growth factor 1 (IGF-1)[19]. IGF-1 is a hormone from the liver that modulates glucose transport in fat and muscle, inhibits glucose output, modulates hepatic glucose production, and lowers blood glucose while suppressing insulin production. IGF binding protein 1 (IGFBP-1) is a specific binding protein that binds to IGF-1 and inhibits insulin-like effects, thus protecting against delayed onset hypoglycemia[19]. IGFBP-1 is not only an important determinant of IGF-1 activity, but also enhances glucose uptake in peripheral tissues and reduces gluconeogenesis and glycogenolysis[19]. Brun and colleagues found that early stages of overtraining may also be associated with reduction in baseline plasma IGFBP-1 concentrations.

Other Factors Influencing BG Concentrations:

In addition to their physiological role in the regulation of blood sugar, glucose-regulatory hormones may fluctuate throughout the day due to circadian rhythm and environmental factors including stress and caffeine. During sleep, BG concentration increases when there is a GH and cortisol peak in the first two h of sleep[22,23]. There is also a rise in cortisol going into the waking h that peaks again just before waking, thereby increasing BG. Stress responses during daytime h have a similar effect. The American Psychological Association (2022) recently reported that seven in ten adults (72%) experience health impacts due to stress[24]. Stress causes an increase in epinephrine and cortisol, which can result in increases in BG. Caffeine also has a similar effect.

Among all caffeinated beverages, a 2020 survey from The National Coffee Association found that coffee is America's second most consumed beverage with 62% of Americans drinking the beverage daily[25]. These data help understand other underlying factors that may result in high BG in people without insulin resistance.

There is speculation that non-nutritive sweeteners (NNS) influence hormone regulation and thus, BG concentrations[26]. This has been hypothesized to be due to the perception of sweetness following consumption of NNSs, which would cause a release of insulin from the pancreas and thus be "mistaken for glucose." This would then increase insulin in the blood and eventually lead to decreased receptor activity due to insulin resistance. Zhang and colleagues investigated this phenomenon in a systematic review and network meta-analysis of acute trials measuring the effect of non-nutritive sweetened beverages on postprandial glycemic and endocrine responses. Trials examined a variety of single NNS (acesulfame potassium, aspartame, cyclamate, saccharin, stevia, and sucralose) and NNS blends (acesulfame potassium + aspartame, acesulfame potassium + sucralose, acesulfame potassium + aspartame + cyclamate, and acesulfame potassium + aspartame + sucralose). Thirty-six trials involving 472 predominantly healthy participants were included, and available evidence suggests that NNS beverages sweetened with single or blends of NNS have no acute metabolic and endocrine effects[26].

Appropriate hydration (e.g., euhydration) is a state of optimal total body water content that is important in maintaining many normal bodily functions, including BG regulation. Dehydration refers to the loss of body water at a rate greater than the body can replace it. A recent review[27] found that dehydration resulted in significantly higher concentrations of blood glucose. This does not mean that there is necessarily more blood sugar in the bloodstream, but rather that the ratio of blood sugar to water has increased. In the acute dehydrated state, however, results are contradictory. One study by Carroll and colleagues[28] (2019) investigated the acute effect of hypohydration on glycemic regulation in healthy adults. 16 healthy adults (8 male, 8 female) participated in the randomized crossover trial design. All underwent an oral glucose tolerance test (OGTT)

when dehydrated and rehydrated after four days of pretrial standardization. One day before the OGTT, participants were dehydrated for one h in a heated tent, followed by fluid restriction or replacement of fluid. OGTT results found that there was no difference in glycemic regulation. Therefore, it can be concluded that an acute bout of hypohydration does not impact blood sugar control in healthy adults[28].

Similarly, Wilson and colleagues[29] evaluated the influence of dehydration on glycemic response around exercise in nine individuals (4 women, 5 men, ages 38±3y) with type I diabetes. Their intervention included a randomized crossover design, with one trial involving progressive dehydration through fluid restriction during and for 2 hours after 60-minutes of exercise, and the other serving as a control with euhydration maintained through water provision. The investigators found that fluid restriction induced a mild dehydration (1% loss of body mass) relative to control, which resulted in elevated post-exercise serum copeptin and cortisol concentration ($p<0.01$). A trend for greater serum glucose concentration during exercise was evident with dehydration and became significant in the second hour of recovery following exercise. Even 48 h following the dehydration trial, there was a significantly reduced prevalence of mild hyperglycemia (28% vs 36% of recorded time) compared with the control condition ($p<0.01$)

CHO Recommendations in Sport:

It has been known for decades that CHO intake can influence exercise capacity. As early as 1924, Levine and colleagues observed that reductions in BG concentrations were associated with impaired performance in marathon runners[4]. Shortly after, Gordon and colleagues[30] conducted a follow-up study in runners from the 1924 race who had BG concentrations below the normal range. They then advised this group to consume a moderately high carbohydrate diet during their upcoming training season. They also studied another group from the same 1924 race which had developed weakness and hunger at any point throughout competition; these symptoms began between miles 14 and 15 of the 25-mile race. This group was then advised to eat a “large amount” of CHO before the race and to consume candies and sugar tea throughout the course in the 1925 race.

Collectively, the results of these interventions found that strategized CHO consumption allowed for maintenance of normal BG concentration and striking improvements in physical conditions during competition. These early studies underpin the importance of appropriate timing and volume of CHO fueling in competition.

Since these discoveries, CHO fueling has received a great deal of attention because of the importance as the main fuel for the brain and central nervous system as well as a versatile substrate for muscular work where it can support exercise over a large range of intensities due to its utilization by both anaerobic and oxidative pathways. As intensity of exercise increases, the need for CHO feeding does as well. Rose and colleagues[31] illustrate this concept in a study investigating leg glucose uptake at rest and during cycle ergometer exercise at different power outputs (65 Watts (W), 130W, and 200W) in 40-minute exercise bouts. During the first 10 minutes of each bout, muscle glucose uptake nearly doubled from 65W to 130W and then again from 130W to 200W. During exercise, the major metabolic fate of blood glucose after entry into skeletal muscle cells is glycolysis and subsequent oxidation[31].

Research has also shown that performance may be limited both by depletion of CHO stores in muscle and liver, the latter of which can result in low blood sugar during exercise. For example, Davis and colleagues[32] investigated the effect of carbohydrate drinks to delay fatigue during intermittent, high intensity cycling in active men and women. 16 young and physically active, but untrained individuals (9 male and 7 female) of comparable activity level, participated in this study. Participants completed one practice trial and two experimental sessions separated by one week. Sessions involved repeated 1-min cycling bouts on a bicycle ergometer at 120-130% VO_{2max} separated by three min rest until fatigue. Participants ingested CHO or placebo (P) beverages (4 ml/kg/BW) immediately before exercise (18% CHO) and every 20 min during exercise (6% CHO). In the CHO group, plasma glucose and insulin were higher, RPE by legs was lower, and time to fatigue was longer in CHO than P. These data suggest the importance of CHO consumption on performance.

Current recommendations for CHO fueling around exercise were developed from extensive research in these areas and attempt to account for the variability of body, timing, competition, etc. CHO fueling becomes more complex around competition due to the variabilities of body mass, timing, competition intensity, type of CHO, and digestion (e.g., glycemic index [GI]). Pre-competition CHO consumption should be around 10 to 12 g/kg body mass in the overall diet for 36 to 48 h before competition[32,33]. Closer to the event (about three to four h), a meal with 3 to 4 grams of CHO per kilogram of body weight is suggested [32,33]. Alternately, one to two h before an event, 1 to 4 g/kg body mass is recommended depending on intensity [32,33] (see *Table 2.2*). During competition, athletes should ingest up to 60 to 90 g/h of well absorbed CHO (i.e., those with a high glycemic index as will be discussed in the next section) such as glucose, glucose polymers, and glucose-fructose mixes for exercise sessions that are over 90 minutes[1]. For events lasting under 90 minutes, 30 to 60 g/h is recommended.

Post-competition, the main goal of CHO nutrition is the rapid recovery of liver and muscle glycogen stores tailored to the upcoming training sessions or competition schedule. Current recommendations suggest athletes ingest moderate to high GI foods as soon as possible at a rate of 1 to 1.2g/kg BM/h for the first 4 h post-exercise when rapid replacement of glycogen restoration is required[1]. Research suggests that consumption of CHO from various sources such as glucose-fructose mixtures are effective at replacement of muscle glycogen. For full repletion of muscle glycogen 24 to 36 h are required, whereas only 11 to 25 h are needed for liver glycogen stores[1]. After glycogen repletion, it is then recommended to resume a normal diet reflecting daily fuel needs based on the energy demands of a sport[1]. Given these recommendations, it is apparent that there is a need for individualization of CHO fueling by sport and personal training goals. Individualization through monitoring glucose could be used as a tool to gain leverage on performance in endurance sports.

GI has proven to be a vital component of CHO fueling around exercise due to metabolic differences of various GI foods and their proceeding performance outcomes [14,33]. GI is defined as the area under the 2h curve of blood

glucose after the ingestion of a set amount of carbohydrate compared with ingestion of the same amount of carbohydrate from a reference food (white bread or glucose)[35]. The lower the GI, the slower the digestion and absorption of that food, whereas the higher the GI the faster the digestion and absorption. As explained in *Table 2.2*, low GI foods may be beneficial before exercise to help stabilize blood glucose, whereas high GI foods may be beneficial during and after exercise when more rapid sources of carbohydrate are needed. While summary of research supporting benefits of consumption of CHO foods with specific GI are beyond the scope of this literature review, the GI is important because it is a direct measurement of the rate at which BG concentrations change based on the properties of CHO is consumed. In a competitive environment, the GI of different carbohydrates can affect performance. For example, one study by Kaviani and colleagues[36] investigated the effects of pre-exercise feeding of a low-GI lentil-based sports nutrition bar with a high-GI bar on metabolism and performance during a simulated soccer match (high-intensity intermittent exercise). Results suggested that during a 90-minute soccer simulated soccer match, a low-GI lentil-based sports nutrition bar provided more metabolic and performance benefits than a high-GI nutrition bar. This included a stabilized BG and insulin concentration, a lower CHO oxidation rate, and a modest improvement in agility running and jumping height. These data emphasize the importance of the timing and type of the CHO consumed leading up to competition.

Table 2.2 summarizes the general guidelines for consumption around competition. Following recommendations by body weight is important to prevent hypo- and hyperglycemia concentrations in BG, which could impair performance during competition.

Table 2.2: CHO Periodization Based on Demands of Upcoming Exercise

	Exercise Intensity	Moderate ~ 60% $VO_{2\ max}$	Heavy ~ 80% $VO_{2\ max}$	Very High > 85% $VO_{2\ max}$
Exercise Duration				

< 90 minutes	*Before*		Low GI foods; 1-2 g/kg; 1-4 h before	Low GI foods; 2-4 g/kg; 1-4 h before	Commencing exercise session with sufficient muscle glycogen stores is essential (low GI foods); 2-4 g/kg; 1-4 h before
	During		No CHO required during	CHO intake recommended is CHO availability before session is limited (high GI foods); 30-60 g/h	Aggressive feeding not recommended; smaller quantities and higher GI including mouth rinsing advised; 0-30 g/h or mouth rinsing
> 90 minutes	*Before*		Low GI foods; 2-4 g/kg; 1-4 h before	Low GI foods; 3-4 g/kg 1-4 h before	Low GI foods; 3-4 g/kg; 1-4 h before
	During		High GI foods; 30-90 g/h	High GI foods; 60-90 g/h	High GI foods; 60-90 g/h

Source modified from Thomas et al.

History of Glucose Monitoring:

Monitoring blood glucose is important in managing both type 1 and type 2 diabetes and preventing short- and long-term complications from hypo- and hyperglycemia. In these conditions, blood glucose regulation is challenged by absence of insulin production and the reduced cellular response to insulin, with type 1 and type 2 diabetes, respectively. Thus, various glucose monitoring methods have been developed to help maintain blood concentration in these clinical populations.

The evolution of these methods began as early as the mid-1800s when there were attempts to quantify glucose in the urine to lay the foundation for modern diabetes care[37,38]. Urine testing, however, did not prove to be effective for monitoring glycemia because it only detects extremely high blood sugar because the extra glucose

in the bloodstream is usually only removed via the kidneys at BG concentrations of about 180 mg/dl and above[38]. Thus, glucose generally isn't excreted via urine if blood sugar is normal or slightly elevated.

After the failure of urine methods to effectively regulate blood glucose, invasive methods of measuring blood glucose became part of standard treatment for individuals with diabetes[16]. These methods detect glucose concentration biochemically in urine or blood samples using a strip or substrate containing oxidizing enzymes. The concentration of blood glucose detected from a strip can then be tracked in association with carbohydrate intake and insulin delivery if applicable and used to help control BG within the normal range. The first glucose meter was utilized in 1970 with Dextrostix, but this product required a large drop of blood and lacked precision and accuracy[37]. By 1980, the Dextrometer was launched which allowed for self-monitoring of blood glucose (SMBG) available on a digital display. Improvements that involved use of smaller blood quantities via fingerstick, electrochemical strips, and new enzymatic testing methodologies continued into the 1990s and 2000s. As described, with blood there is a need for a method of drawing blood known as "finger-pricking." Clinical patients are burdened with the responsibility of frequently monitoring their BG concentrations with finger-pricking, which can result in complications that include infections or loss of sensitivity due to scarring and callus formation[16,39]. This can be seen as a barrier that may cause patients to inconsistently monitor their glucose throughout the day and potentially miss critical fluctuations in concentration. Other limitations regarding invasive monitoring methods include the quality of an enzymatic strip and patient user error[39].

Recent advances in BG monitoring technology have allowed for the development of CGMs, which measure BG concentration using ISF by implanting a sensor into the subcutaneous layer of the skin. In 1999 Medtronic released the Minimed (Medtronic Diabetes, Northridge, CA) the first U.S. Food and Drug Administration approval of the first "professional" continuous glucose monitor (CGM)[36]. The Minimed recorded patients' glucose readings every ten seconds with average readings reported every five minutes. Sensors could be worn for up to 72 h but relied on repetitive sensor calibration with a finger stick glucose sample every 6 to 12 h. Until

recently, all CGM devices required calibration with fingerstick blood glucose measurements. The first `real-time " CGM was the Glucowatch Biographer (Cygnus, Redwood, CA)[37]. This device was worn as a wristwatch using “reverse iontophoresis” to stimulate the secretion of subcutaneous fluid, from which glucose was measured using an electrode. The Glucowatch was not a commercial success due to site irritation from the sensor wire. In 2004, Medtronic (Northridge, CA) introduced the Guardian Real-Time CGM system, which notified users of potentially dangerous hyperglycemia or hypoglycemia[37]. Since then, other companies including Abbott [Almeda, CA] and Dexcom [San Diego, CA]) have released their own models of real-time CGMs.

Although interstitial fluid is less invasive, there are complications such as time lag, irritation, and cost that are still being researched to further improve comfort, accessibility, and accuracy of BG monitoring. Therefore, depending on personal preference, individuals who monitor BG either utilize blood, interstitial fluid, or both. *Table 2.3* summarizes the advantages and disadvantages of use of blood versus interstitial blood sugar for glucose detection. This includes a review of the properties of each bodily fluid and what affects their reliability for monitoring.

Table 2.3: Advantages and Disadvantages of Common BG Monitoring Bodily Fluids

	Advantages	Disadvantages	Time lag (min)
Blood	- Most consistent measurement - No time lags - Standardized procedures for obtaining blood samples via venipuncture	- Invasive and painful - Typically obtained via finger prick; few alternative sites for obtaining - Blood sugar is not a static measurement, the finger-stick only reads a moment in time	None

Interstitial fluid	- Reduction of finger-pricking - Trends in glucose can be identified - Easier and less invasive identification of night-time hypoglycemia - Data can be uploaded and shared with others - Data supports decision-making and awareness relating to glucose control - Devise provides alerts when glucose is too low or high	- Cost - Data overload can confuse or worry some users - Time lag - Possible sensor problems relating to insertion, skin irritation, or loss of adherence - May still require finger-prick calibration (certain bands) - Constant presence of sensors on the body	- 5-15

Data summarized from Podlogar et al., McMillin et al., and Enter et al.

CGM Technology:

CGM technology captures glucose concentrations in real-time with 1- to 15-minute measurement intervals[1]. ISF is the fluid that fills spaces between the blood vessels and surrounding cells and is composed of water, amino acids, glucose, fatty acids, coenzymes, hormones, neurotransmitters, salts, and cellular products). ISF can be used as a monitoring alternative to blood due to the "push-pull" phenomenon. This is when glucose is pushed from the blood into the ISF space at times of increased BG, and conversely, when glucose is pulled from the ISF to the surrounding cells when BG concentration is decreasing[40]. This push-pull phenomenon allows BG dynamics to be frequently captured.

CGM devices contain three main components: a sensor, a transmitter, and a receiver. Typically, the device consists of a small pager-like monitoring device that receives a signal from a sensor filament that is less than 0.4 mm thick inserted into the subcutaneous layer[40]. To read ISF, this sensor creates a signal using the glucose oxidase reaction, which produces a current that is proportional to the glucose concentration in the ISF. Calibration algorithms are used to convert the signal into a BG concentration by comparing the signal detected from a known concentration of glucose[40]. These less invasive methods for blood glucose detection have enabled

a new paradigm in glucose monitoring, which now gives individuals the ability to monitor the progression of their glucose concentration more easily over time.

The evolution of the CGM started in the late 1990s, with the development of the first hard-wired CGM[41]. In 2000, a wireless and real-time CGM was developed, which allowed users to constantly "view" their data[37]. Throughout 2010 to 2015 the first 14-d sensor with intermittently scanned CGM was available, cloud-based, and "shareable" data. In 2016, the first fully implantable CGM was developed with a 90-d sensor[37]. The 2020s brought a widespread integration of CGM data into insulin pump devices for automated insulin delivery for type 1 diabetes, as well as the approval of the first "sports" CGM[40].

There are currently four companies that have personal CGM devices on the US market, Abbot, Dexcom, Medtronic, and Senseonics. Some of the most recent releases include the Dexcom G7, Freestyle Libre 2 and 3 (Abbot), Medtronic Guardian 4, and Eversense (Senseonics). *Table 2.4* provides details on the functionality of each device.

Table 2.4. Current CGMs on the Market and their Characteristics

	Dexcom G7	**Libre 2**	**Libre 3**	**Guardian Connect or Guardian 4**	**Eversense**
Display device	Smartphone or receiver insulin pump	Smartphone or reader	Smartphone	Smartphone or insulin pump	Smartphone
Maximum wear time	10 d	14 d	14 d	7 d	180 d
Warm-up time	30 minutes	1 h	1 h	Up to 2 h	24 h
Calibration required	0	0	0	0	Two per d for 21 d, then once per d
FDA-approved sites	- Abdomen - Upper buttocks	- Upper arm	- Upper arm	- Upper arm - Abdomen - Upper buttocks	- Upper arm

Drug interactions	- Hydroxyurea	- Vitamin C	- Vitamin C	- Hydroxyurea - Acetaminophen	- Tetracycline antibiotics

Data summarized from Miller et al., Asker Jeukendrup, https://www.dexcom.com/get-started-cgm/214?sfc=7014y000001eDPGAA2&gclsrc=aw.ds&gad_source=1&gclid=CjoKCQiAh8OtBhCQARIsAIkWb6-8GUgAGxPo6im6wGPyAhB22-hHFk102p3PnGMpbATv9H6kCHrKGK4aAn6uEALw_wcB-, https://www.freestyle.abbott/us-en/products/freestyle-libre-3.html, Medtronicdiabetes.com., https://www.ascensiadiabetes.com/eversense/

Contributing Factors to Inaccurate CGM Readings:

As CGMs are still in development, many factors may interfere with accurate readings of BG via CGM. These include medications and vitamins as shown in T*able 2.4* as well as dehydration. Hydroxyurea, which is a medical treatment for patients with myeloproliferative disorders and hematologic conditions (i.e., sickle cell disease), and acetaminophen, which is a common over-the-counter pain medication, cause readings to be higher than the actual glucose concentration. CGMs are not recommended for individuals who take hydroxyurea[46,47]. Acetaminophen, however, is only a concern when taken over a dose of 1 gram every 6 h. Acetaminophen taken at this dose increases ISF concentrations of acetaminophen glucuronide, which is detected as glucose by CGM sensors[48,49]. Similarly, vitamin C could cause false- high sensor glucose values. Cho and colleagues identified that Vitamin C is a strong antioxidant that inactivates free radicals and can be oxidized at the surface of electrochemical strips producing electrons and increasing the current[48]. Along with this, Abbot informs their users that taking over 500 mg of ascorbic acid (vitamin C) falsely raises glucose concentrations. In contrast, aspirin, and tetracycline antibiotics cause artificially low readings of glucose concentrations[47, 48]. Aspirin contains salicylates, while the exact mechanism is not fully understood, salicylates may influence the chemical composition of the interstitial fluid, affecting the sensor's ability to accurately measure glucose levels and, ultimately, decreasing them. Multivitamin and mineral supplements, on the other hand, are not currently thought to pose a concern on the CGM reading as they don’t contain a dose high enough in any one vitamin or mineral to potentially alter readings[30,31]. Overall, these findings suggest that both medication and supplement use should be considered when analyzing glucose concentrations from individuals who utilize CGMs.

Dehydration also may interfere with CGM readings by causing false low readings. Data suggests this could decrease the glucose supply within the interstitium, resulting in glucose levels that are lower when measured in the interstitial compartment compared with either capillary or venous measurements. Therefore, ISF glucose measurements under hypohydration may not be reliable[46,47].

CGM in Sport:

Although little testing has been done on CGMs in the physically active, non-diabetic population, there has been increasing application in the sports setting[1]. The ability to understand and utilize the BG concentrations in real-time is becoming a newfound component that may possibly contribute to the success of training and competition in sports via individualization of CHO consumption[14]. This is suggested to help athletes through increasing performance, managing stress, monitoring nutrition, speeding recovery, identifying LEA status, and training as BG can reflect metabolic and inflammatory conditions[13]. As recently reviewed by Jeukendrup, CGM data may be useful for sport to (1) study the glycemic response to a meal (2) help prevent "bonking" or "hitting a wall," in sports training or competition from the depletion of glucose, and (3) support cognitive performance during sport due to the high impact of glucose on cognitive function. Jeukendrup refers to CGMs as a "personal nutrition coach" that can help detect hypoglycemic events in sport before it's too late and tell an athlete[13,41].

A recent study from Coates and colleagues[50] emphasizes the benefits that CGMs may provide in nutrition and monitoring during training. Using CGMs, they measured altered carbohydrate oxidation during exercise in overreached endurance athletes. Results demonstrated a reduction in CGM-derived glucose and carbohydrate oxidation during submaximal exercise in overreached athletes.

Additionally, CGMs also allow for nocturnal BG monitoring for athletes[5]. This data is insightful because fluctuations in blood glucose concentration may have negative effects on athletes' function (e.g., recovery of glycogen stores and cognitive function/performance)[5]. Using CGM to see fluctuations of BG concentration in

athletes could thus be used to potentially individualize strategies to counter episodes of impaired glycemic control in real-time. Research needs to undergo more sophisticated investigations of high-performance athletes in real-world events and with real-world nutrition strategies.

A recent study from Bowler and colleagues emphasizes CGMs use in measuring blood glucose concentrations in athletic populations[6]. They aimed to use CGM data to develop athlete-specific reference ranges for glycemic variability under standardized diet and exercise conditions. Results from this investigation provided insight on glycemic variability in the athletic population and the different methods to assess CGM data efficiently and effectively.

Methods of Assessing CGM Data:

CGMs gather large amounts of data from the frequent sampling of glucose (every 1 to 15 minutes) across a 24-h period, and for multiple days. Reviewing and interpreting this large volume of data can become difficult, particularly when data is collected over long periods of time. Many methods have been developed to decrease this barrier in CGM utilization. For individuals with diabetes, Abbott offers a database called "LibreView" where CGM data can be uploaded to and then easily accessed at any time[51]. LibreView also interprets the data for users into standardized reports that are easy to understand by comparing values to standardized time in ranges based on reference recommendations of the American Diabetes Association (ADA)[51]. Example of data from these reports include glycemic variability, ambulatory glucose profile (AGP), daily glucose profiles (average glucose, time in target range, time below target range, time above target range). The glucose range is automatically set at 70 to 180 mg/dL but this can be set to different ranges.

For individuals without diabetes, Bowler and colleagues suggest the use of glycemic variability formulas to better understand the standard deviation interquartile ranges, mean of daily differences (MODD), and mean of amplitude of glucose excursions (MAGE)[6]. MAGE quantitates major fluctuations in glucose within a 24- h

period, while MODD complements these values by calculating the mean differences between glucose values on consecutive days at the same time[6]. Higher MODD values indicate larger difference in day-to-day whole body glycemia and have been associated with complications in individuals with diabetes[6]. Some of these complications include increased oxidative stress, endothelial dysfunction, and cardiovascular events. *Table 2.5* explains how to utilize these methods.

Previous studies of frequent blood or interstitial glucose concentration have analyzed blood glucose concentrations during sleep[52] and the area under the curve responses following meals or exercise[50,53,54]. Most of current data of these findings were conducted on the diabetic population. Even in studies that utilize CGM to monitor glucose concentrations in athletes, there is little information on methods to interpret CGM data in a detailed manner. To date, there is only one recent study (Bowler, 2024) in athletes and one in non-athletes (Hill, 2011) that utilize MAGE and MODD to measuring glycemic variability for CMG data analysis. Therefore, it will be important to investigate more methods where CGM data can be easily interpreted for the athletic population.

Table 2.5: Methods of Measuring Glycemic Variability

Measure of glycemic variability	Description	Formula	Variables
MAGE	Interprets major fluctuations in whole-body glycemia	MAGE = $\sum \lambda/\chi$ if $\lambda > v$	λ = blood glucose changes from peak to nadir χ = number of valid observations v = 1 SD of mean glucose for a 24- h period
MODD	Interprets between-day variability in glucose	MODD = $\sum_{t=t_1}^{t_k} \frac{\sum_{t=t_1}^{t_k} G_t - G_{t-1,440}}{k}$	k = number of observations with an observation 24 h ago BG = glucose measured t = time (in minutes)

Source modified from Bowler et al.

Low Energy Availability in Sport:

LEA is a state in which the energy intake is too low, or exercise expenditure surpasses the dietary intake, which results in an insufficient amount of energy to maintain normal physiological function (i.e., metabolic, and immune function, bone health, menstrual cycle)[55]. EA can be defined as the difference between energy intake and energy expended during exercise relative to fat-free mass and is energy remaining for normal physiological function. It is calculated as EA = dietary intake (kcal) - exercise energy expenditure (kcal) / fat-free mass (FFM) (kg)[55]. Research supports[7,55] that having an EA value of less than 30 kcal/kg FFM for women and approximately 9 to 25 kcal/kg FFM for men puts them in a LEA condition. Studies[11,55,56] suggest that athletes are particularly more at risk of experiencing LEA compared to the non-athletic population, especially those engaging in sports with high energy expenditure (e.g., running, swimming, cycling). The prevalence of LEA in athletic groups is estimated to be 22% to 58%, depending on the sport[5]. Several studies[46,56] have emphasized the prevalence of LEA among runners, particularly female runners. This is most likely because running has high physiological demands, high training load, and is a weight-sensitive sport [46,56]. With runners, LEA can be attributed to the culture of sport (i.e. the belief that "lighter is faster" with body weight), underlying body image issues, and societal pressure to attain a certain body type or aesthetic appearance[54]. Notably, 79% of women were at risk for developing LEA, compared to 54% of men[56]. LEA may also be unintentional due to decreased appetite, inadequate cooking skills, or a busy schedule[11].

Whether intentional or not, LEA poses detrimental consequences to health and performance. Some of these maladaptation's include endocrine alterations, suppression of reproductive hormones, and altered metabolic responses. These can vary in severity depending on the duration of LEA status[12]. Hormones affected by LEA (i.e., insulin, cortisol, and GH) may alter glycemia. There is preliminary evidence that interstitial glucose decreases in response to severe energy restriction[5,9].

Discoveries of this correlation date to 2003 from Loucks and colleagues[57]. They investigated luteinizing hormone pulsatility disruption at a threshold of energy availability for five days in the early follicular phase.

Participants included 29 regularly menstruating, habitually sedentary, young women of normal body composition. Subjects expended 15 kcal/kg of lean body mass (LBM) per day in supervised exercise at 70% aerobic capacity while consuming a commercially available product (e.g., Ensure) to set energy availability at 45 (control) and either 10, 20, or 30 kcal/kg LBM/d in two randomized trials separated by at least two months. Blood was drawn via a venous catheter at 10 min intervals for a 24- h period. The study found that participants in the treatments of 10, 20, and 30 kcal/kg LBM/d had reduced mean fasting plasma glucose concentrations by 15%, 5% and 3%, respectively. Reductions in plasma glucose occurred during both the feeding and fasting phases when energy availability was at 10 kcal/kg LBM/d, whereas they only occur during the fasting phase when energy intake was at 20 and 30 kcal/kg LBM/d.

Three years later, another study from Loucks[58] investigated the response of luteinizing hormone pulsatility to 5 days of low energy availability. The study population included healthy, habitually sedentary, young women of normal body composition with five to eight y (n=9) and 14 to 18 y (adults, n=10) of gynecological age. Participants were randomly assigned to receive energy availability at 45 (control) versus 10 kcal/kg of fat-free mass per day for five days in the early follicular phases of separate menstrual cycles. Results suggest that central glucose was a key regulator of gonadotropin hormone-releasing hormone (GnRH) and thereby, LH pulsatility.

In 2016, Smith and colleagues[59] investigated LEA by characterizing interstitial glucose concentration and hypoglycemia using CGM (Medtronic Minimed). The intervention consisted of a 2-d calorie deficit with sustained exercise. Motivation for this study was to investigate the effects of commonly experienced short-term energy deficits in populations of athletes and military personnel. Short term energy deficits can result in degraded physical and cognitive performance due to severe hypoglycemia. Participants included 23 young volunteers (17 male, 6 female) of normal weight. They increased habitual daily energy expenditure by approximately 1,600 kcal/day through prescribed exercise. Along with this, participants consumed diets

designed to either maintain energy balance or induce a 93% energy deficit. Results suggest that interstitial glucose concentrations were approximately 18 mg/dL lower during energy deficit intervention versus energy balance. The percentage of time spent in mild hypoglycemia was higher during energy deficit compared to energy balance whereas time spent in severe hypoglycemia was not different between interventions. These data suggest that severe hypoglycemia rarely occurs in healthy individuals that endure severe, short-term energy deficits secondary to heavy exercise and inadequate energy intake.

Another study in 2016 from Koehler and colleagues[60] researched LEA in exercising men and the association with reduced leptin and insulin. Participants included 6 exercising men that were randomized to undergo two conditions of LEA (15 kcal kcal/ kg FFM/d) and two energy-balanced conditions (40 kcal/ kg FFM/d). During one low energy availability and one balanced condition, participants exercised to expend 15 kcal/ kg FFM/d; no exercise was conducted during the other two conditions. After both LEA conditions, leptin and insulin were reduced. These reductions were not influenced by the exercise condition.

In 2023, Hutson and colleagues[9] investigated how jumping mitigates short term effects of LEA on bone resorption in nineteen young regularly menstruating females. Participants underwent two 3-d conditions providing 15 and 45 kcal/ kg FFM/d of energy availability. In the LEA condition, participants were randomized to either a jumping or no jumping protocol. Jumping protocol involved 20- high impact jumps twice per day during LEA. Before and after each intervention β-CTx, P1NP, and total triiodothyronine (T3), glucose, calcium, magnesium, and phosphorus were measured via serum. Data reported brief bouts of high impact jumping, performed twice daily in the morning and evening, may help to mitigate a rise in bone resorption and reduce bone loss within the first three days. There was also a significant decrease in fasting plasma blood glucose from day one to day four in the 45 kcal/ kg FFM/d group.

These data, along with other studies[5,9] emphasize that LEA may disrupt normal glucose homeostasis. This suggests the importance of potentially utilizing CGMs as a tool of an early detection device for LEA athletes. However, there is a need to further investigate the utility of interstitial glucose values from CGMs in this population as an early marker of disturbed metabolic function secondary to LEA [5,9].

Theoretical, Technical, and Practical Limitations of CGM:

The complex nature of using ISF as a marker of BG regulation during exercise and at rest needs to be considered and more fully investigated before CGMs are utilized as a tool in sports. Many theoretical, technical, and practical limitations need to be considered and are summarized in T*able 2.6*.

Theoretical Limitations:

CGMs cannot currently identify glucose concentrations that denote optimal CHO availability or differentiate optimal and suboptimal CHO intake practices[5]. As data is interpreted, it will also be important to understand and validate the logic behind the data for the utilization by sports nutritionists, coaches, and athletes in meaningful ways[6]. Bowler and colleagues suggest that it is likely that athletes who are unfamiliar with CGM data would attribute post-exercise hyperglycemia as a response to a particular strategy of CHO intake rather than viewing it as a normal response to intense exercise[6].

There is concern that the utilization of CGMs for individuals with LEA could pose the risk of furthering the complications of LEA or the development of disordered eating patterns. Considering CGMs can provide interstitial glucose as often as every minute, there is a concern with "glucorexia" which (becoming over-sensitive to small, non-physiological swings in interstitial glucose values)[5].

Technical Limitations:

Timing is a critical component of sports and regulating BG. Data suggest that the time lag of CGMs could be an issue for some sporting situations. Although blood and ISF are intricately connected, there is a delay in glucose detection in ISF and may not directly reflect BG[60]. Glucose concentrations in one compartment change rapidly (e.g., during exercise) and it takes can take approximately 1 to 15 minutes until the same change can be measured in the other compartment due to the slow diffusion of glucose[61].

Current limitations for use in sports and other free-living environments include CGM sensor durability, software design, accuracy, reliability, usability, and influence of environmental variables (i.e., water immersion, extreme climates, sweat, and physical contact with others during sports training, and competition)[6,41]. Additionally, the adhesive film (which keeps the CGM reader in contact with the surface of the skin) has the potential to deteriorate when immersed in water; limiting the utilization of these tools for water-based athletes (i.e., swimmers, triathletes, kayakers, water polo, rowers, etc.). Along with this, temperature, humidity, and sweat can also reduce the sensor durability due to excess moisture, which prevents the transmission of data from the sensor to CGM receiver[6,41]. Along with these concerns, there is a potential for the CGM to be pulled out in team or combat sports, especially ones that may require uniforms or protective garments.

Accurate CGM readings are also dependent on a normal hydration status[46,47]. We know that dehydration interferes with CGM readings by causing false low readings. ISF glucose measurements under hypohydration may not be reliable because of lacking the amount of fluid necessary to measure ISF [46,47]. Achieving an appropriate hydration status during competition is extremely difficult, especially in endurance sports where you can lose as much as three liters of fluid in an h of intense exercise, while having little to no time allotted for rehydration during competition[62]. Considering. CGM readings are inaccurate when dehydration is present, this becomes a significant limitation for the utilization of CGMs in sports.

Jeukendrup indicates that there is still uncertainty if CGMs are truly fuel sensors because the source of glucose during exercise comes from both blood and stored glycogen within skeletal muscle glycogen[13,41]. Therefore, blood glucose concentrations do not fully depict carbohydrate energy sources. Similarly, there is a limitation of GI as a practical tool due to the potential of different responses from the same food between any two individuals. The variety of meal and food combinations makes the tracking of changes in BG concerning CHO intake more complicated. There is no clear understanding of how to track multiple GI values of combination snacks and meals[13,41]. Lastly, measuring only BG concentration provides no information on the flux, or rate at which glucose is entering or exiting circulation or is therefore being produced or consumed.). Glucose flux can vary significantly from one person to another despite stable BG and could diminish the effectiveness in the application of CGM data as a "fuel sensor"[13,41].

Practical Limitations:

The cost of CGMs and subscriptions to the supporting mobile applications poses a limitation for the use of this tool. Along with the commercial viability, there is also a need for approval for non-diabetic, individual use in competition that may vary by country. For example, the approval for the use of CGMs in targeted sports was not granted by the Therapeutic Goods Administration in Australia. Similarly, the rules from international and national organizations present a challenge for CGMs. Examples include the no needles policy for the Australian Institute of Sport or the banning of the use of physiological monitoring of glucose and lactate within sanctioned races for the International Governing Federation of Cycling[6]. These factors collectively limit the use of CGM to athletes in some countries.

Table 2.6: Limitation of CGM Use in Sport

Theoretical Limitations	Technical Limitations	Practical Limitations
- New-user confusion with unfamiliarity of normal BG concentration fluctuations - "Glucorexia"	- Timing delay - CGM accuracy, durability, reliability, usability in a competition environment with external factors - Not inclusive to water-immersion sports	- The expense of CGMs and subscriptions supporting mobile - Rules vary from country to country during competition (i.e., no

- Potential to induce disorder eating patterns	- Excess sweat and/or humidity may cause sensor to fall off - Hydration status is influential to readings; optimal hydration is extremely difficult during competition - Uncertainty if CGMs are a true fuel sensor; glucose sources during exercise come from both blood and stored glycogen within skeletal muscle glycogen - Measuring only BG concentration provides no information on flux (i.e., rate) of glucose entering and leaving	needle policy for the Australian institute sport)

Data summarized from Logue et al., Bowler et al., Asker Jeukendrup, https://www.mysportscienceacademy.com/course/cgm-in-sport, Merino et al.

Previous Studies Using CGMs in Active, Non-Diabetic Populations

Assessing the accuracy and validity of CGM data is necessary to ensure this tool is effective for implementation in athletic populations. To date, however, few studies have been conducted on the effectiveness of GCM in the active, non-diabetic population.

Clavel and colleagues[63] evaluated the concurrent validity of CGM systems at both rest and in association with a high-intensity interval training session in eight non-diabetic recreational athletes. As a secondary analysis, they also assessed the effect of two different, standardized breakfasts (CHO vs protein) on CGM validity. Participants performed interval training on a bike and BG was monitored every 10 min using two different CGM devices (FreeStyle Libre and FreeStyle Optimum) as well as finger-prick BG measurements over 4 time periods (post-breakfast, pre-exercise, exercise, and post-exercise). The data were analyzed using the Bland-Altman method (standardized mean bias), mean absolute relative difference (MARD), and Clarke Error Grid (EGA). The results suggest that mean bias was small at post-breakfast, pre-exercise, and post-exercise but was more variable during exercise. Similarly, there was a higher median absolute relative difference observed during exercise. Along with this, there was no effect of breakfast composition on the MARD results. The authors concluded that the CGM devices examined should only be used at rest, or after either a CHO or protein-rich breakfast, but recommended they not be used during exercise because of decrease accuracy.

Thomas and colleagues[64] inserted two Ipro2 CGM devices (Medtronic Minimed, Northridge, CA, USA) into the abdomen of a young female athlete. The intervention included two, 2- h continuous exercise bouts in the fasting state followed by provision of a post-exercise glucose bolus of 60 g CHO, which were carried out three days apart. On rest days, BG was measured four times a day before meals and sleeping to calibrate the device. During exercise, reference BG measurements were taken regularly. This was every 10 min during the two h of exercising, every five minutes for 30 minutes after the glucose solution was ingested, then one final measurement taken 40 minutes after the glucose solution was ingested. These tests were then repeated when the athlete was known to be sedentary. The data suggested that CGM devices agreed well with each other and with reference measurements during vigorous exercise. During rest, CGM accuracy was reduced compared to the reference measurements, but the accuracy between the CGMs remained. The lower accuracy during sedentary conditions was attributed to the less active pumping of ISF, which like blood, relies on muscle movement to circulate and mix. Thus, contradictory to Clavel and colleagues, the authors concluded that CGMs are more accurate during vigorous movement due to the rapid mixing and equilibrium between blood and ISF.

Another recent study from Coates and colleagues[65] investigated sensor location on the effectiveness of glucose monitoring during exercise in nine healthy participants (3 women, 6 men). The group evaluated CGM data collected from the active muscle of the leg (vastus medialis) versus the traditional sensor placement on the arm (triceps brachii). Data was collected following 100g of glucose ingestion during 30-minute experimental trials of rest (control), graded cycling, electrically stimulated quadricep contractions, and passive whole-body skin heating. Finger capillary glucose was used to assess sensor accuracy. They found that at rest, glucose measured from the leg was consistently lower than in the arm, whereas during graded cycling, leg glucose was lower only after maximal intensity exercise. In contrast, there were no differences between sensors during quadriceps stimulation. Overall, findings indicate that at rest, arm placement of a CGM is ideal for data collection. This is because findings determined that during passive or active leg-muscle contraction, site-specific differences

compared to capillary samples are attenuated [37]. Therefore, CGM placement on the leg is a better alternative to CGM location on the arm to obtain more information on athlete glucose flux during cycling exercise.

In 2019, Doering and colleagues[53] conducted a study measuring repeated muscle glycogen supercompensation with four days' recovery between exhaustive exercise. Participants included seven trained cyclists (6 male and 1 female). The study involved a 9-day experimental period with three intermittent exhaustive cycling trials on days one (trail 1), five (trial 2), and nine (trail 3). Following trial one, cyclists were fed a high carbohydrate diet for eight days. Trials consisted of two min work intervals interspersed with two min recovery, which were repeated until exhaustion. Each 72- h period between trial days included two days of low volume cycling and a rest day. Resting muscle glycogen and total work completed was determined for each trial day. Blood glucose concentrations were measured using a CGM (CGM; iPro2; Medtronic, Northridge, CA, USA) inserted into the abdominal region, as well as blood glucose concentrations obtained from capillary blood (Abbot, IL, USA) for CGM calibrations prior to meals and sleep. A second CGM was inserted on day four of the experimental period. Results suggested that baseline muscle glycogen on day one was super compromised on day five and again on day nine. Total cycling work capacity increased from trial one to trial two; a larger effect was observed in trial three compared to one. 72 h interstitial glucose AUC was lower throughout the second CHO loading period compared to the first CHO load, despite matched dietary intakes. While this study showed that back-to-back muscle glycogen supercompensation is possible following exhaustive exercise with four d of high carbohydrate feeding, it also demonstrated that CGMs can be used simultaneously with other measurements to appropriately measure the CHO fueling needs of an athlete.

A study published in 2020 by Ishihara and colleagues[66] investigated the application of CGM for assessment of individual carbohydrate requirements during ultramarathon races. Participants included seven ultramarathon runners (4 male and 3 female). The goal of this study was to evaluate the feasibility of utilizing CGMs (Abbot, Freestyle Libre) to improve the carbohydrate intake of ultra-marathon runners for competition, specifically

aiming to determine the minimum carbohydrate requirement to maintain blood glucose concentration and race speed during ultramarathons. The intervention was an observational study during the 2019 Ultra trail Mt. Fuji (165 km). Glucose profile and average running speed of the top five finishers for each sex were recorded, as well as self-reported food and drink intake were monitored during the race. Glucose profile was divided into 11 segments by timing gates. Results suggest that glucose concentration obtained from CGM positively correlated with running speed segmented and that CGMs could be a practical tool to guarantee optimal carbohydrate intake for individual ultra-marathon runners.

Similarly, Kinrade and colleagues[67] (2021) observed the diets of ultra-endurance runners prior to and during a field-based 24- h trail race where participants covered as much distance as they could in 24- h. Then, they compared observations from race participants with current recommendations for CHO intake and multiple transportable carbohydrates (MTC's). Pre-race diet (each 24 over 48 h) was recorded via weighted food records and included the pre-race meal (one to four h pre-race) and in-race diet food consumption during the 24 h event). Heart rate and interstitial glucose concentrations were also recorded. CHO intake over the 24 to 48 h pre-race was found to be lower than recommended, although pre-race CHO consumption was within recommended range. In-race CHO intake, however, was only in the 30-60g/h range with suboptimal amounts of MTC's consumed. Along with this, moderate to strong positive correlations were observed between the distance run during the race and both CHO and energy intake in each of the three diet periods studied.

Sengoku and colleagues[68] published a study investigating the relationship between glucose profile and change in running speed in two ultra-endurance runners. The intervention included inserting a CGM (Medtronic, Minimed) into the subcutaneous abdominal tissue 35 h before a 100-km race. Glucose profile and CHO consumption were monitored until the end of the race. Runner A maintained normal blood glucose concentrations completed throughout the duration of the race the race and a less of a race pace decrement despite consuming less CHO (249 g) than runner B. Runner B (recreational runner) had greater glucose

concentration variability and experienced a rapid decline in glucose (hypoglycemia) near the end of the race, despite a higher CHO consumption than runner A (366 g). These results suggest that elite ultramarathon runners may have the ability to prevent large decreases in BG concentration regardless of the amount of energy intake during the race.

Francios and colleagues[69] examined the blood glucose responses and other physiological responses during a multi-day expedition-style adventure race in eight competitors (2 female, 6 male). The race was titles the GODZone and included trekking, mountain biking, kayaking and canoeing; ~500 km in four to seven d. CGMs (Medtronic Ipro2 CGM with an Enlite Sensor) were inserted in the abdomen in all participants and data was measured throughout the race. Body mass, urinary solutes, blood pressure, and heart rate during resting, standing, and repeated squat-stand conditions were assessed pre and post. Data reported that Competitors experienced higher variability in blood glucose and lower glucose concentrations. Blood pressure during resting, standing, and squat-stand conditions was significantly lower. There was also increased glycemic variability and more frequent periods of low BG levels. Hypotension also observed in competitors.

In 2023, Coates and colleague [50] published an investigation on the use of CGMs to evaluate carbohydrate oxidation during exercise in overreached endurance athletes. The study included nine endurance athletes: five male, three female. Participants underwent a 5-week training block consisting of one week of reduced training, three weeks of high-intensity overload training, and one week of recovery training. At each time point, a submaximal cycling test and 5 km time-trial was performed 15 min after ingestion of a 50 g glucose beverage with glucose recorded each minute via CGM. Results show a suppressed power output for the five km time-trial and a reduction in CGM-derived glucose and carbohydrate oxidation during submaximal exercise during the overreached training state compared to baseline data from week one. This data provides insight on the validity of utilizing CGM as a tool for detecting overreached athletes.

The most recent study on the application of CGM in sport is from Bowler and colleagues in 2024[70]. They investigated the utilization of CGMs to measure interstitial glucose in athletic populations. This was inspired by their aim to develop athlete specific reference ranges for glycemic variability under standardized diet and exercise conditions. 12 elite racewalkers (7 men and 5 women) completed two 4-d trials separated by 4-d where athletes were provided a high-energy, high carbohydrate diet and completed standardized daily exercise. Timing of food consumption and exercise was consistent among the 4-d trials. Data were analyzed using MAGE, MODD, and standard deviation to find that CGM derived glycemic variability in endurance athletes are like like those of healthy individuals, despite the variation in activity and energy intake.

Table 2.7: Previous Studies of CGM utilization in Endurance Sporting Events

Author (year)	Participants	Purpose	CGM Model	Site of Sensor
Sengoku (2015)	N=2 male well trained/trained ultra-marathon runners.	Investigate the relationship between glucose profile and the change in running speed	Medtronic Minimed	Abdomen
Thomas (2015)	N=1; female, 23 y, BMI: 21/kgm^2, resting HR: 50 beats per minute (bpm), training 10-17 h/week	Investigate the accuracy and performance of CGM devices in an active athlete	Medtronic Minimed Ipro2	Abdomen
Francois (2018)	N=8 trained adventure racers; 2 female, 6 male	Examine the blood glucose and other physiological responses during a multi-day expedition-style adventure race.	Medtronic Ipro2 CGM with an Enlite Sensor	Abdomen

Doering (2019)	N=7 trained cyclists; 6 male, 1 female (VO2peak: 57 ± 4 mL kg^{-1} $min^{-1)}$	Determine whether a 4-d period of high carbohydrate intake can supercompensation muscle glycogen and exercise work capacity on back-to-back occasions	Medtronic Ipro2	Abdominal region
Ishihara (2020)	N=7 ultramarathon runners; 4 male (41.5 ± 6.2 y; 172.9 ± 2.7 cm; 66.0 ± 9.3 kg; BMI: 22.2 ± 2.8 kg/m^2; LBM: 56.3 ± 5.9 kg; fat mass: 22.2 ± 2.8 kg) and 3 female (42.6 ± 1.2 y; 158.0 ± 6.5 cm; 47.9 ± 3.8 kg; BMI: 18.9 ± 0.7 kg/m^2; LBM: 40.7 ± 4.3 kg; fat mass: 18.9 ± 0.7 kg)	Evaluate the feasibility of CGMs to improve the carbohydrate intake of ultramarathon runners using a CGM. Determine the minimum carbohydrate requirement to maintain blood glucose level and race speed during ultramarathons	Abbott FreeStyle Libre	Upper arm
Clavel (2022)	N=8 nondiabetic recreational athletes (30.8 ± 9.5 y; 173.6 ± 6.6 cm; 70.3 ± 8.1 kg)	Assess the concurrent validity of continuous blood-glucose-monitoring system (CGM) post breakfast, pre-exercise, exercise, and post exercise, while assessing the impact of 2 standardized breakfasts on the observed level of validity	Abbott FreeStyle Libre	Non-Dominant upper arm (i.e., back of triceps brachialis)

Kinrade (2021)	N=18 amateur ultra-endurance runners (41.5±5.1 y; 75.8±11.7 kg)	Examined the dietary intake of amateur ultra-endurance runners prior to, and during a 24- h ultra-endurance event	Abbot Freestyle Libre	Subcutaneous tissue layer of the upper arm
Coates (2023)	N=9 healthy participants; 3 female and 6 male (27 ± 5 y; 77.0 ± 12.7 kg; BMI: 25.0 ± 3.3 kg/m^2; VO_{2max}: 47.8 ± 9.7 $ml.kg^{-1}.min^{-1}$)	Evaluate whether CGM sensors worn on the active muscle may provide enhanced insight into glucose control in nondiabetic participants during cycling exercise compared to traditional sensor placement on the arm.	Abbott Libre Sense	Triceps brachii versus vastus medialis
Coates (2023)	N=9 endurance athletes; 5 male, 3 female (27 ± y; 77.0 ± 12.7 kg; BMI: 25.0 ± 3.3 kg/m^2 ;VO_{2max}: 47.8 ± 9.7 mL kg^{-1} min^{-1})	Investigate whether carbohydrate utilization is altered during exercise in overreached endurance athletes and examine the utility of continuous glucose monitors to detect overreaching status.	Abbott Libre Sense	Subcutaneous fat pad over the triceps brachii

Bowler (2024)	N=12 elite racewalkers; 7 male, 5 female (22.4 ± 3.5 y; VO_{2max} 61.6 ± 7.3 mL kg^{-1} min^{-1})	Develop athlete-specific reference ranges for glycemic variability under standardized diet and exercise conditions	Abbott Freestyle Libre 2	Back of upper arm

Data summarized from Coates et al, Doering et al, Clavel et al., Thomas et al., Coates at al. (2023), Ishihara et al., Sengoku et al, Francios et al., Bowler et al.

Chapter 3. Manuscript

Characterizing the Use of Continuous Glucose Monitoring During Experimentally Induced Short-Term Low Energy Availability in Female Endurance Runners

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1. Introduction

Female athletes in endurance sports are among the highest risk for developing low energy availability (LEA) with 22 to 58 % at risk depending on the sport[5]. LEA is defined as a state in which the dietary intake is too low, or exercise expenditure surpasses the dietary intake, which results in an insufficient amount of energy to maintain normal physiological function (i.e., metabolic, and immune function, bone health, menstrual cycle)[55]. Research supports[7,55] that having an EA value of less than 30 kcal/kg FFM/day puts women in a condition of LEA. Several studies[46,56] have emphasized the prevalence of LEA among runners and, particularly, female runners. Consequences of LEA include compromised physiological processes such as decreased bone health, gastrointestinal and cardiovascular dysfunction, disrupted glucose homeostasis, and irregular menstruation; all of which can contribute to impaired sports performance or injury risk[5,10]. Finding more effective ways to mitigate or detect LEA before becoming prolonged could be the next step in elevating women's health and performance.

CHO has long been known as the most vital fuel source for athletes' exercise performance; adequate CHO optimizes training, delays fatigue, and promotes recovery[1]. Low CHO availability can be a detriment to the performance endurance athletes[2]. Metabolically, this is related to insufficient liver and muscle glycogen concentration resulting in a fall of blood glucose (BG)[3]. To fully understand the importance of CHO and proper fueling for athletes, it is also important to understand blood glucose (BG) homeostasis to facilitate optimal physiological conditions for performance; BG homeostasis is central to the success of an exercise bout. Studies

of these findings date back to 1924 when Levine and colleagues[4] found that athletes who experienced lower BG (hypoglycemia) had a decrease in performance. To combat hypoglycemia, athletes were recommended to consume a higher CHO diet before the event or consume CHO candy during the event. Even with this insight, proper fueling continues to be an issue across many sports on an individual basis[5,6].

Previous studies have observed reductions in morning fasting blood glucose after two to five days of LEA[, 8, 9, 57, 58, 60, 67]. These and other studies, however, required assessment of BG via catheter insertion or via regular obtainment of blood glucose by fingerstick. More recently, studies[50, 53, 63, 65, 66, 67, 68, 69] have attempted to utilize continuous glucose monitors (CGMs) as a mode for assessing BG in athletic populations even though these tools were originally designed to help control BG in individuals with insulin-dependent and non-insulin-dependent diabetes. CGM's are inserted into interstitial tissue and sample interstitial fluid (ISF) glucose every 1 to 15 minutes. Interstitial glucose serves as a marker of BG with an estimated 10-to-15-minute delay from real-time BG concentrations. Preliminary evidence from Smith and colleagues[59] in male athletes suggests that CGM ISF concentrations also decrease in response to two days of LEA. Considering these data, we speculate that CGMs could be used as an early detection device for LEA risk in athletes.

Little data is presented on the use of CGMs of athletic populations and no study to date has evaluated LEA in female endurance runners utilizing a CGM. Therefore, the purpose of this study is to characterize the CGM data in this population during conditions of LEA and evaluate the use of CGM as a tool for detecting LEA conditions in athletes at higher risk for developing LEA.

2. Methods

2.1. Overview of Study Design

The current study is a secondary analysis of a study that evaluated the effect of impact **l**oading on bone biomarkers in energy-restricted female runners (LOAD). The LOAD study is a randomized cross-over

intervention with the primary objective of determining the effect of short-term, high-impact loading on biomarkers of bone remodeling in trained, female, long-distance runners in the presence of LEA[71]. Participants underwent 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). A computer-based random number generator for research (randomizer.org, Version 4.0) was used to randomize the participants into the condition order. Participants completed two, 5-d experimental conditions (day one to five) separated by at least one menstrual cycle. EA of 15 kcal/kg FFM/d on day one to five was achieved through dietary restriction (EI = 30 kcal/kg FFM/d) and exercise (EEE = 15 kcal/kg FFM/d) consisting of daily treadmill running at 65-70% VO_{2max} with jumping exercises (RUN+IL) or without (RUN). Experimental conditions began during the follicular phase of the menstrual cycle one to seven days after the onset of menses. CGM sensors (Freestyle Libre Pro, Alameda, CA) and a smart watch (venu SQ, Garmin International, Olathe, KS, USA) were employed to assess interstitial glucose concentration and physical activity was monitored using smart watch technology (venu SQ, Garmin International, Olathe, KS, USA). Primary outcomes of the intervention are reported elsewhere[69] and include biomarkers related to bone formation and resorption. This master's thesis will utilize secondary data collected as part of LOAD, which will focus specifically on glycemic data collected from the CGM in participants who had complete data from the RUN arm. Data from the RUN+IL were not included because of the possibility that the impact loading exercises, which involved high impact jumping, would influence 24- h glycemic responses. The outline of the study procedures is shown in Figure 3.1.

Ethical approval for this study was obtained from the Institutional Review Board at Virginia Tech (IRB #22-168). Participants provided written informed consent before participation.

2.2. Participants

Participant were recruited via utilizing flyers, social media, and digital communication. Eligible participants were naturally menstruating female runners (with self-reported normal menstrual cycles of 24 to 32 days) between 18 to 35 y of age with a body mass index (BMI) between 18.5 to 30 kg/m2 and were non-smokers, not using hormonal contraceptives, not pregnant or lactating, and were free from bone injury within the previous 6 months. Individuals with low bone density (z-score < -2), low hemoglobin (< 12.0 g·dL−1), abnormal thyroid function (thyroid stimulating hormone < 0.4 mIU·L−1 or > 5.5 mIU·L−1)[72], and those who had recently recovered from an eating disorder within the last 12 months, or used medications that would affect study results (e.g., corticosteroids, anticonvulsants, gonadotropin-releasing hormone agonists) were excluded from study participation. Eligibility was confirmed using a standardized health history questionnaire, pregnancy test by urine, blood biochemistry, and body composition assessment by dual-energy x-ray absorptiometry (DXA) during the initial screening visit. Participants enrolled were considered low risk for existing LEA according to the Low Energy Availability in Females Questionnaire (LEAF-Q; score <8), which is a validated screening tool for LEA in female endurance athletes[73].

2.3. Screening and Baseline Testing

Before study enrollment, participants underwent screening and baseline testing that involved completion of questionnaires and a screening blood draw to ensure eligibility followed by anthropometric and VO_{2max} testing (Figure 3.1). Blood was drawn by venipuncture and was analyzed for a complete blood count (CBC) and thyroid stimulating hormone (TSH); to be eligible participants had to have hemoglobin and TSH within the normal limits as outlined above.

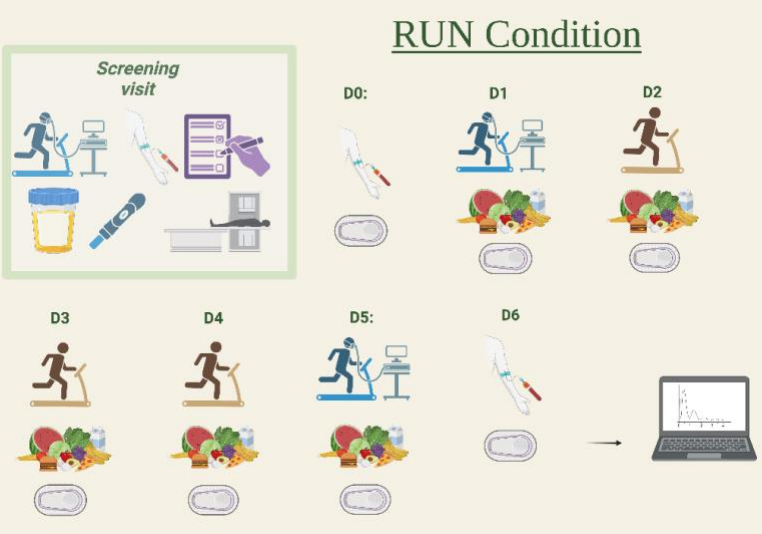

Fig. 3.1

Questionnaires: Participants were asked to complete a standard health and medical health questionnaire, which was 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. As previously mentioned, participants also completed the Low Energy Availability in Females Questionnaire (LEAF-Q) during the first screening visit to assess risk for existing low EA. The LEAF-Q has been validated as a screening tool for LEA in female endurance athletes[71].

Anthropometrics and Body Composition: Participants reported to the lab for the first screening visit after an overnight fast (no-caffeine) with participants also instructed not to engage in heavy exercise for 36 h before testing and to adequately hydrate the evening before. Height was measured to the nearest 0.1cm using a stadiometer (Welch Allyn Scale-Tronix 5002, Milwaukee, WI, USA) and total mass (TBM) was measured to the nearest 0.1kg on a digital physician's scale (WB-110A NETP III, Tanita Corporation of America Arlington Heights, Illinois USA). Fat mass (FM), LBM, body fat percentage (BF%), bone mineral content and bone mineral density were assessed using a fan-beam DXA (Lunar iDXA, enCORE Version 15, General Electric Healthcare, Madison, WI, USA). The DXA scanner was calibrated daily before the scanning sessions according to manufacturer's instructions. All DXA scans were performed and analyzed by the same licensed DXA

technician to ensure consistency. Participants wore the same lab-provided T-shirt and shorts for optimal scanning conditions.

*Urine sample and pregnancy test: P*articipants provided a small cup of urine immediately before the DXA at baseline. The urine was evaluated for hydration status via urine specific gravity (USG) using a handheld analog refractometer (Fisherbrand, Thermo Fisher Scientific, USA). Euhydration was considered less than 1.020 $g \cdot mL^{-1}$ [172]. A pregnancy test was also performed on the urine sample and those which tested positive were not enrolled in the study.

Aerobic capacity (i.e., VO_{2max}): A graded exercise test was performed on a treadmill to assess aerobic fitness via indirect calorimetry (Parvo Medics TrueOne 2400). Heart rate was measured during the test by a heart rate strap and sensor (Polar H10, Kempele, Finland). The test began with a 5-minute warm-up at 0% grade and at a speed predetermined by the participants. Following the warm-up phase, the workout load increased each minute by increasing treadmill speed or grade by 0.5 mph or 2.5%, respectively until the participant could longer continue or volitional exhaustion was reached. This entire exercise testing protocol lasted 12 to 20 minutes. To qualify as an acceptable maximal test, participants had to meet two of the four following criteria: (1) a leveling or plateau of VO_{2max} (defined as an increase of < 2 $mL \cdot kg^{-1} \cdot min^{-1}$ with increased workload); (2) RER ≥ 1.10; (3) maximum heart rate within 10 beats of age predicted maximum [208 - (0.7 x age)] [30]; (4) rating of perceived exertion (RPE) ≥ 17. Participants had to have a VO_{2max} of > 35 mL/kg/min to be included in the study.

2.4. Baseline Diet and Exercise

Habitual diet was assessed using food intake recorded on three consecutive days including two weekdays and one weekend. Food records were analyzed by a registered dietician (TS) using ESHA's Food Processor® Nutrient analysis software (version 11.9.14, EHSA Research, Salem, OR, USA). In addition to the 3-d food

record, participants wore a Garmin smartwatch (venu SQ, Garmin International, Olathe, KS, USA) to assess heart rate, step count, exercise duration, and estimate energy expenditure during structured exercise and play.

2.5. Experimental Conditions

2.5.1. Dietary Procedures

Participants were provided controlled, weighed diets equal to 30 kcal/ kg FFM/d). Energy intake was manipulated individually based on FFM measured via DXA. Menus consisted of three meals and one snack of similar whole foods and commercial products that provide approximately 55% of total calories from carbohydrates, 20% protein, and 25% fat. Diet was modified based on participants' allergies or preferences (i.e., lactose intolerance, vegetarian, etc.) within reason. Participants were encouraged to eat meals and snacks at the same time every day. A daily multivitamin (25 mcg of vitamin D3 as cholecalciferol, 160 mg of calcium as calcium carbonate, 18 mg of iron as ferrous fumarate; Nature Made, West Hills, CA, USA) was provided during the five days of energy restriction. Participants were instructed to take the multivitamin with a meal of their choice and record the time of consumption on the mealtime log. All prepared and returned food items were weighed to the nearest 0.5 g to estimate actual intake. Caffeinated beverages were also allowed as long as they did not have any calories.

2.5.2. Running Procedures

Running Economy was assessed on the first and last day of each experimental condition at the start of each supervised treadmill run. Heart rate was measured during the test by a heart rate strap and sensor (Polar H10, Polar Electro, Kempele, Finland). The test began with participant running for 4-minutes at three moderately easy speeds of 5.5, 6.0, and 6.5 mph. The last two minutes of oxygen consumption and carbon dioxide production data were be used to determine metabolic economy (mL oxygen consumed per kg bodyweight per minute relative to the set work performed). This test lasted 12 minutes. Following culmination of the economy

test, the workload was increased to 65% VO_{2max}. Oxygen consumption was measured for the first 4 to10 minutes of the steady state run.

Participants underwent supervised exercise sessions consisting of treadmill running on five consecutive days. On the first day, exercise energy expenditure (EEE) was measured using indirect calorimetry (ParvoMedics) during a controlled "titration" run to help determine running speed at 65% VO_{2max}. A heart rate monitor was used simultaneously to measure heart rate. Total EEE for exercise sessions was set at 15 kcal/kgFFM/d. From this, the running protocol (duration) needed to expend or "burn" a total of 15 kcal/kgFFM/d was then determined and used throughout the study. Running time on the treadmill varied based on participants body weight, percent body fat, VO_{2max}, and running efficiency. Treadmill duration was determined based on expenditure of 15 kcal/kg FFM/d while running at an intensity of ~65% VO_{2max}. Participants were instructed to refrain from all physical activity outside of the supervised exercise sessions unrelated to daily activities (e.g., getting dressed, walking to car).

2.5.3. Blood Biochemistry

Fasting blood samples were collected by venipuncture after an overnight, 10- h fast. A 20 mL blood sample was collected on day zero and day six to measure insulin and cortisol concentrations. Serum specimens clotted at room temperature for at least 15 minutes but no longer than two h before centrifugation. Plasma specimens were kept on ice before centrifugation. Specimens were centrifuged at 3500 rpm for 13 minutes at 4°C (Centrifuge 5804R 15amp, Eppendorf, Enfield, CT, USA). CBC, TSH, parathyroid hormone (PTH), ferritin, total iron binding capacity (TIBC), iron, cortisol, and insulin were analyzed the same day at a commercial lab (LabCorp Burlington, Burlington, NC, USA). Aliquots of plasma and serum were stored at -80°C until analysis at the Virginia Tech Metabolism Core (Virginia Tech, Blacksburg, Virginia, USA).

2.5.4 CGM Devices

Participants' glucose was measured continuously throughout the intervention using digital CGM devices from Abbott Laboratories (Freestyle Libre Pro, Alameda, CA). Monitors were placed the day before initiation of the LEA condition towards the back of one of the upper arms and covered with 3M Tegaderm adhesive film (Two Harbors, Minnesota) for improved durability. The arm selected was dependent on either participant preference or avoiding the arm predicants were likely to sleep on. The Freestyle Libre Pro samples the glucose concentration from a small amount of interstitial fluid (< 1 microliter) every 15 minutes (96 times per day) throughout the 5-d experimental conditions and blinds participants to their glucose readings. The CGM was removed on day six and data was gathered using the monitor sensor and then downloaded via a universal serial bus (USB) to a password secured application (LibreView) for further data analysis.

2.6. Statistical Analysis

Glucose data from the 5-d condition was analyzed to obtain average 24- h glucose, average glucose during sleep, average fasting glucose, time in target (TIT), time above target (TAT) time above (TBT), the glycemic variability (GV) as percent coefficient of variation, mean amplitude of glucose excursions (MAGE), and mean of daily differences (MODD). The target range was set according to the typical reference blood glucose concentration of 70 and 120 mg/dL [10] with TIT, TAT and TBT calculated as a percent of time over a 24- h period from 12 AM to 11:59 PM. Analysis for these variables were calculated by the "LibreView" free-access software (Abbott, Alameda, CA) where CGM data can be uploaded to and then easily accessed at any time[50]. These data were then downloaded into an excel document in 15-minute intervals for analysis. Average glucose during sleep was calculated from all data points between the h of 10 PM and 8 AM; whereas average fasting glucose was estimated from values between 2 AM and right before the first meal of the day. MAGE and MODD were calculated using the equations of Hill and colleagues[73]. Specifically, MAGE was calculated using a software created by Hill and colleagues (EasyGV; Nathan R Hill. © University of Oxford 2010) for each day of the intervention, while MODD was measured between days one and two, two and three, three and four, and four and five and then calculated utilizing a macro formula on excel. Data was summarized as a mean ± one standard deviation. Data was analyzed using repeated measures analysis of variance to determine the effect of LEA day on glucose concentration and glycemic variability; post hoc comparisons were performed using paired t-tests. The significance level was set a priori at $p<0.05$. All statistical analysis was conducted using IBM® SPSS® Statistical software (Version 28.0.2.2 (14), IBM Corporation, NY, USA).

3. Results

Participant Characteristics

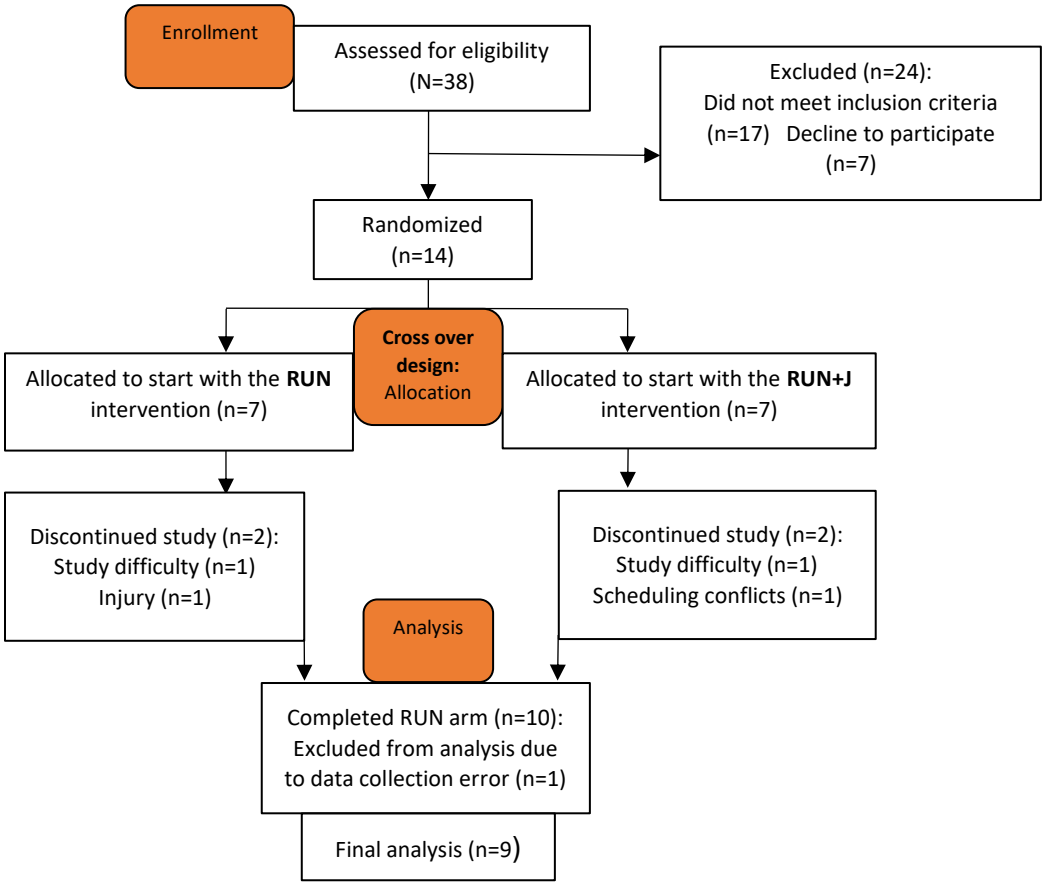

Fig. 3.2

As summarized in Figure 3.2, 38 participants were initially assessed for study eligibility. Of the 38 screened, 24 were excluded due to not meeting inclusion criteria or declining to participate. Fourteen were then randomized into the first intervention period. Ten of which who completed the RUN arm, were eligible for inclusion into this sub analysis; however, one participant had incomplete data due to poor CGM reading, leaving a final sample of nine. Among the nine with CGM data, one experienced a rash in the shape if a ring which was surrounding the CGM by day six of the intervention.

Table 3.1 describes the characteristics of participants that completed the RUN arm. Participants considered themselves well-trained and reported training a minimum of two to three times a week. Given these criteria,

four participants were in the tier one athlete category and five in tier two based on recent classification framework developed by McKay and colleagues[74]. All data analysis forward-looking depicts the findings from participants described in Table 3.1 from the RUN arm of the LOAD study.

Table 3.1 Participant Screening Characteristics

Characteristics	White (8), Mixed (1)	
	Mean ± SD	**Range**
Age (y)	26.9 ± 4.0	20.8 - 31.4
Height (cm)	170.19 ± 6.3	158.1 - 177.8
Weight (kg)	65.3 ± 9.3	48.8 - 76.2
BF (%)	28.6 ± 5.8	21.4 - 38.8
Fat Free Mass (kg)	47 ± 4.7	37.6 - 52.5
VO_{2max} (ml/kg/m)	44.6 ± 7.3	37.0 - 55.6
TSH (uIU/mL)	1.63 ± 0.7	0.97 - 3.3
Hemoglobin (g/dL)	13.18 ± 0 .7	12.10 - 14.2

Dietary Control, Controlled Running, and Energy Availability

Table 3.2 summarizes participant habitual kcal and CHO consumption and the intervention prescription for kcal and CHO based on provision of 30 kcal/kg FFM/d and 55% percent of energy as CHO. Prescription energy intake (kcal) was significantly lower in the LEA diets compared to baseline (P=0.002). The relative amount of CHO intake, however, was not significantly lower than habitual CHO intake in participants (P=0.193).

Table 3.2 Habitual and Prescription Diet

	Habitual		**Prescription**	
Participant ID	**kcal**	**CHO (g/kg)**	**kcal (30 kcal/kg FFM)**	**CHO (g/kg TBM)**
03	1819	2.8	1549	3.0
04	2295	5.2	1129	3.2
07	1663	3.2	1365	3.3
08	1872	3.1	1380	2.8
09	3087	5.7	1573	3.1
10	1925	2.8	1429	3.0
12	1823	3.1	1539	2.8
14	1656	1.4	1434	2.6
16	2190	5.1	1292	3.2

Table 3.3 Participant Baseline VO_{2max} and Intervention Exercise Prescription

Participant ID	Baseline VO_{2max} (ml/kg/min)	Prescription			
		Exercise Energy Expenditure (kcal)	Run speed (mph)(m/s)	Run Duration (minutes)	Intensity (%Vo_{2max})
03	38.8	678.6	5.7 (2.55)	82	65.6%
04	54.8	530.5	6.8 (3.04)	60	65%
07	55.6	632.6	6.7 (2.99)	63	64.7%
08	45.3	651.36	5.2 (2.32)	61.3	73%
09	48.5	735.6	5.5 (2.46)	71.4	66.7%
10	38.1	618.3	4.9 (2.19)	80	71.3%
12	37.3	708.1	4.5 (2.01)	82	68.3%
14	37	671.4	4.5 (2.01)	68	77.2%
16	46.4	598	5.9 (2.64)	73.3	69.6%

Note: *Values from D1 and D5 were not included in this Table due to the variability from the economy and titration tests compared to their exercise prescription)*

Table 3.3 summarizes the baseline absolute and relative percent VO_{2max} ran during the exercise prescription, exercise energy expenditure (kcal), run speed, and run duration for each participant during day two through day four of the intervention. Participants completed the intervention at an average VO_{2max} intensity of 69% ± 4.2 with a run speed of 5.5 mph (2.4m/s) ±0.85 and run duration of 71.2 ± 9.3 minutes. Energy expenditure during exercise averaged 649.7 ± 66.8 kcal.

Interstitial Glucose Concentration

Table 3.4 Interstitial Glucose Concentration via CGM

	D1 mean ± SD	D2 mean ± SD	D3 mean ± SD	D4 mean ± SD	D5 mean ± SD	P-value (time effect)
24- h Average (mg/dL)	85.8 ± 7.9[b, d]	89.6 ± 9.8[a]	89.4 ± 8.9	88.3 ± 7.9	91.1 ± 8.3[a]	*0.025
Glycemic Variability (mg/dL)	21.3 ± 3.6	18.2 ± 3.4	18.5 ± 4.4	21.9 ± 6	21.6 ±3.6	0.14
Time In Target (%)	76 ± 10[b]	85 ± 12[a]	86 ± 15[d]	78 ± 15[c]	83 ± 10	*.03
Time Below Target (%)	19 ± 13	10 ± 12	9 ± 17	17 ± 16%	11 ± 12	0.052

Time Above Target (%)	4 ± 6	5 ± 5	5.8 ± 5	4.7± 4	6 ± 5	0.17
Fasting BG (mg/dL)	79.5 ± 11.2[c]	78.4 ± 9.7[c]	70.1 ± 10.2 [a, b]	73.5 ± 9.8	79.2 ± 19.6	*0.04
Sleeping BG (mg/dL)	81 ± 10.4	82.9 ± 11.3[c]	75.3 ± 10.1[b]	77.3 ± 8.5	81.6 ± 18.9	0.07
MAGE	53 ± 12.8	51.2 ± 16.1	46.7 ± 13.2	50 ± 14.3	49.2 ± 13.4	0.85
		TBTvs2 mean ± SD	**D2vs3 mean ± SD**	**D3vs4 mean ± SD**	**D4vs5 mean ± SD**	
MODD		15.5 ± 4.3	14 ± 3.3	13.7 ± 3.2	15.1 ± 3.4	.695

** Significant time-effect, [a] Significant compared to D1, [b] Significant compared to D2, [c] Significant compared to D3, [d] Significant compared to D4, [e] Significant compared to D5*

As shown in Table 3.4, average 24- h glucose concentration and TIT significantly increased from day one to day five (P=0.025 and P=0.03, respectively). There was also a significant time-effect for fasting BG (P=0.04) Although sleeping BG over the 5-d intervention was not significant, there was a significant decrease from day one to day three (P=0.002). Fasting and sleeping BG had the same trends; the concentrations of both fell from day one to day three, followed by an increase by day five that were like those of day one. Additionally, there was not a significant time-effect for TBT, TBT, glycemic variability, MAGE, and MODD all had an insignificant time-effect (P>0.05).

Blood Hormone Concentrations

Table 3.5 Pre and Post Intervention Hormone Concentrations

	Insulin (U/mL)		**Cortisol (ug/dL)**	
Participant ID	**Pre-Intervention**	**Post-Intervention**	**Pre-Intervention**	**Post-Intervention**
03	12.8	4.4	19.2	20.9
04	4.2	2.1	22.7	19.2
07	4.6	1.9	16.3	23.1
08	5.8	1.1	12	11.8
09	4.4	5	4.4	19
10	4.3	3.6	19.2	12.6
12	8.2	7.4	19.9	24
14	3.1	7.8	22.6	19.4

16 Mean ± SD	3.3 5.6 ± 3.08	1.4 3.8 ± 2.5	8.8 17.9 ± 4.7	18.5 18.7 ± 4.1

The glucose regulatory hormones (i.e. cortisol and insulin) measured at D0 and D6 are summarized in Table 3.5. Average concentrations of both hormones did not change from pre- to post-intervention *(P*>0.05).

4. Discussion

It has been shown that during short-term periods of LEA, there is a decline in BG concentrations. Previous studies have primarily revealed this by measuring BG concentrations via venipuncture after a morning fast[5, 8, 57, 68]. This study is the first to characterize glucose concentration during short-term experimentally induced low energy availability in female endurance runners using a CGM. By analyzing data from a primary investigation that induced LEA under tightly controlled conditions[71], we found that five days of LEA of 15 kcal/kg FFM/d progressively increased 24- h BG as well as presented a noticeable trend in fasting and sleeping BG concentrations where participants had a significant decrease in BG from day one to day three of LEA, but then adapted to the intervention conditions after day three to obtain concentrations similar to the first day of energy restriction (day one).

Previous studies in the sports and exercise arena have evaluated the use of CGM as a possible tool for use during exercise in non-diabetic individuals[7]. Specifically, these studies have monitored the effect of carbohydrate consumption on interstitial glucose concentration[53], determined the best cite for sensor location (upper arm vs. active leg muscle)[65], and attempted to better understand carbohydrate utilization and glycemic variability in athletes[69] including during conditions of overreaching[50]. To our knowledge, no studies have yet utilized CGMs to better understand the impact of LEA in female long-distance runners.

In the current study, average fasting and sleeping interstitial glucose concentrations were mostly in agreement with previous studies that have observed decreases in fasting blood [57, 58, 68] and interstitial glucose [59] concentrations after several days of LEA. Average interstitial glucose concentrations during fasting and sleep

declined across the first three days, followed by a rebound in these concentrations from day three to day five. Surprisingly, and contrary to our hypothesis average 24- h BG concentrations progressively increased across the five days. We speculate that this discrepancy is likely due to alterations in glucose production versus utilization that are driven by decreased insulin and/or increased BG counterregulatory hormones. Although we did not see differences in fasting insulin or cortisol on the morning of day six, following five days of LEA, compared to the morning of day one, we can speculate that the combination of dietary and exercise induced stress from such conditions may have induced adaptations in BG concentrations possibly due to increases in epinephrine, cortisol, glucagon, human growth hormone (HGH) at least during the day, which cause an increase in gluconeogenesis and substrate utilization of glycerol and glucogenic amino acids. These alterations may be unique to this population undergoing a LEA intervention versus previous studies in athletes.

To our knowledge, the equations from Hill and colleagues as markers of glycemic variability (e.g. MAGE and MODD)[75] have not been thoroughly investigated in the non-diabetic, athletic population. These values may help shed light on the influence of LEA on BG regulation. As compared to a recent study from Bowler and colleagues[70], who aimed to develop athlete specific reference ranges for glycemic variability under standardized conditions, MODD averaged 12.6 ± 1.8 mg/dL (0.7 ± 0.1 mmol/L) and was similar to our results which did not change throughout the duration of the intervention and ranged from an average of 13.7 to 15.5 mg/dL. Both were also in agreement to the normative reference ranges for non-athletic participants without diabetes of 14.4 ± 24.2 mg/dL (0.8 ±1.4 mmol/L). In contrast, MAGE averaged 36.0 ± 5.4 mg/dL (2.0 ± 0.3 mmol/L) and 25.2 ± 12.6 mg/dL (1.4 ± 0.7 mmol/L) in the studies of Bowler et al[70] and Hill et all[75], respectively. These values were lower than our average MAGE throughout the 5-d intervention average of 50 ± 14 and also did not change across time. These findings suggest that a state of LEA seemed to influence variability within a single day based on MAGE (calculated as the mean height of excursion greater than one standard deviation) but not between days given that MODD estimates the average difference between glycemic values at the same time on different

days. As MAGE and MODD did not change over time, this additionally suggests that additional daily variability was evident within the first day of LEA.

We were somewhat surprised that there was a significant time-effect for TIT with no clear pattern. We can speculate that this was also because of BG counterregulatory hormones which adapted to LEA conditions via gluconeogenesis to maintain BG homeostasis. There needs to be further investigation on the appropriate ranges for different populations who utilize CGM as the standard 70 to 120 mg/dL may not be a "one size fits all" to determine TAT, TBT, and TIT. These values, specifically TIT and TAT, may not be pertinent to athletic populations because with intense exercise, BG concentrations increase. When liver releases glucose at a higher rate than it is taken up by muscle and there is a disproportionate increase in plasma epinephrine. As mentioned, this can result in an elevation of BG concentrations during intense exercise that further increases immediately after exercise and can persist for up to one h. As a result, plasma insulin concentration rises post-exercise to restore the elevated glucose concentrations[20]. TBT range should be further investigated in athletic populations considering our understanding of how BG concentrations fluctuate during exercise and states of LEA and how this can affect health and performance.

While the study was very tightly controlled it was limited by lack of a longer baseline during which participants consumed their typical diet and the relatively smaller sample size. Similarly, habitual diet was self-reported and there is a possibility of over or under reporting CHO intake. This may have influenced the significance in CHO consumption from baseline in comparison their prescription diets. Along with this, CGM use with simultaneous obtainment of blood glucose via finger stick and assessment of hydration status may have helped validate use of interstitial glucose as marker of blood glucose concentration). Additionally, more frequent testing of blood-glucose regulatory hormones including insulin, cortisol and glucagon would have helped better understand the pattern of glycemic changes observed over the five days of experimentally-induced low energy availability. We also would like to have measured ketones in the blood before, during, and after the intervention. This would

have helped us understand substrate utilization for each participant and whether their bodies were depleted of glucose stores for energy which would trigger a utilization of fat stores and therefore, ketone concentrations. The lack of diversity in our sample, which was mostly younger Caucasian women, also affects the application of CGMs in sport and should be further investigated among more diverse populations. There also needs to be more research conducted on the appropriate ranges for different populations who utilize CGM as the standard 70 – 120 mg/dL may not be an accurate or appropriate representation of TAT, TBT, and TIT. Finally, we understand the potential benefit of CGMs to identify LEA during a short-term intervention, but we are unfamiliar with CGM derived glycemic patterns in this population during long-term LEA. This limiting factor may be influential in whether the tool could be useful in detecting LEA conditions.

5. Conclusion

Based on these data, CGMs have been proven to be significant in capturing differences in glycemic variability under conditions of short-term LEA. In sports where LEA is more prevalent, the potential usefulness of CGMs to combat the onset of this condition in sport is promising; CGMs will allow us to gain further insight on BG patterns during conditions of LEA. Our study was able to conclude that CGM-derived 24- h average BG patterns in athletes increase over a 5-d LEA intervention. This, combined with our findings of a significant increase in fasting BG, sleeping BG, and TIT provides a better understanding of ways in which athletes, coaches, and other supporting staff could potentially utilize CGMs to enhance overall health and performance of athletes. For CGM utilization to increase in this population, there needs to be further investigation on the implementation this tool.

Ways to catalyze the implementation of CGM in sport includes increasing the accuracy, validity, convenience, and user experience. The ways glucose monitoring industries should aim to do so is by investigating even less invasive forms of monitoring as well as methods that would help decrease the potential for a loss of, inaccuracy,

or invalidity of data in some sports settings (i.e., increasing durability). As new models are being created for the athletic population, they must be regularly tested to appropriately support an athlete's needs.

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APPENDICES

Appendix A: Study 1 Virginia Tech Institutional Review Board Research Protocol

Division of Scholarly Integrity and Research Compliance
Institutional Review Board
North End Center, Suite 4120 (MC 0497)
300 Turner Street NW
Blacksburg, Virginia 24061
540/231-3732
irb@vt.edu
http://www.research.vt.edu/sirc/hrpp

VIRGINIA TECH

MEMORANDUM

DATE: February 14, 2024

TO: Enette Larson-Meyer, Elaina Lynn Marinik, Firoozeh Tarkesh, Anna Morozov, Janet T Rinehart, Trisha Marie Sterringer, Lauryn Faith Mericle

FROM: Virginia Tech Institutional Review Board (FWA00000572)

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

IRB NUMBER: **22-168**

Effective February 14, 2024, the Virginia Tech Institution Review Board (IRB) approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

https://secure.research.vt.edu/external/irb/responsibilities.htm

(Please review responsibilities before beginning your research.)

PROTOCOL INFORMATION:

Approved As:	**Expedited, under 45 CFR 46.110 category(ies) 9**
Protocol Approval Date:	**March 13, 2024**
Protocol Expiration Date:	**March 12, 2025**
Continuing Review Due Date*:	**February 19, 2025**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

ASSOCIATED FUNDING:

The table on the following page indicates whether grant proposals are related to this protocol, and which of the listed proposals, if any, have been compared to this protocol, if required.

Invent the Future

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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

PRINCIPAL INVESTIGATOR:

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FUNDING:

Sponsor(s): no sponsor
Funded already or in the proposal phase?: Proposal phase
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)	Yes

		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)	
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) 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)	Yes
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- h 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 h)

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-h 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- h 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-h 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 all 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:

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

N/A

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

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

21.1 Describe:

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

The data safety monitoring plan (DSMP) for this study focuses on close monitoring by the 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

Appendix B: Study 1 Approved Informed Consent

Title of Research Study: The Effect of Impact Loading on Bone Biomarkers in Energy-Restricted Female Runners (Protocol # 22-168)

Principal Investigator: Enette Larson-Meyer, PhD, RD, FACSM, enette@vt.edu, 540-231-1025

Other Study Personnel: *Trisha Sterringer, MS, RD, CSCS, tsterringer@vt.edu; Elaina Marinik, PhD emarinik@vt.edu*

Key Information: The following is a short summary of our study to help you decide whether or not to be a part of the study. More detailed information is listed later on in this form.

The reason for this study is to determine the efficacy of adding brief, high-impact loading exercises (jumping) to normal running training on protecting bone health over a 5-day period in generally healthy, female long-distance runners eating a low-calorie diet. There will be two, 5-day periods that participants will compete. In one phase, participants will complete 50 jumping exercises in addition to a treadmill run. In the other phase, participants will only complete a treadmill run. Meals and snacks for both 5-day periods will be provided to participants.

Why am I being invited to take part in a research study?

We invite you to take part in a research study because you are a trained, female long-distance runner between the ages of 18-35y. You will be eligible to participate in this study if you have a BMI between 18.5-30 kg/m^2 and have a normal menstrual cycle without the use of contraceptives. Additionally, you are invited to participate in this study if you are able to run on 5 consecutive days for at least 60 minutes each day. You will not be eligible to participate if you have had a bone fracture in the last 6 months, have a recent history of a diagnosed eating disorder, use contraceptives or certain medications that could potentially affect bone metabolism, are pregnant, lactating, or have a thyroid condition.

What should I know about being in a research study?

- Someone will explain this research study to you
- Whether or not you take part is up to you
- You can choose not to take part
- You can agree to take part and later change your mind
- Your decision will not be held against you
- You can ask all the questions you want before you decide

Why is this research being done?

Many long-distance runners struggle to consume enough calories each day to match the number of calories they are burning during exercise and as part of daily living. Undereating for long periods of time is a serious concern because it can have negative effects on general health and sports performance. One of these long-term consequences is related to bone health. With undereating, bone can start to be broken down faster than it can be rebuilt. Even though it can take months and even years for bone to be seriously affected, this may lead to weak and brittle bones later in life if it is left untreated. Typically, athletes are recommended to increase the number

of calories they eat to prevent negative health concerns. However, not all athletes may be willing or able to increase their calories based on their performance goals, nutrition knowledge, or concerns with food security insecurity. This means that alternative strategies need to be investigated to counteract the negative effects of undereating on bone health. This study proposes to evaluate the effect of adding short bouts of high-impact jumping exercises to typical endurance training to promote healthy bone metabolism. This will specifically involve completing two phases of a supervised treadmill run and consuming a reduced-calorie diet (provided by the research team) on 5 consecutive days. In one of the phases, you will also perform 5 sets of 10 jumping exercises on 5 consecutive days in addition to the treadmill run. Results of the study will serve as an important *first step* in helping exercise and medical professionals understand more about how to protect and manage the bone health of female long-distance runners.

How long will the research last and what will I need to do?

The time commitment for this study will be 2 weeks of an experimental trial separated by approximately 3 weeks of your normal lifestyle, in addition to screening and baseline visits (19 days total). This will include: a) a screening visit at the *Human Integrative Physiology Laboratory;* b) a baseline testing visit at the *Nutrition and Exercise Metabolism Laboratory*; and c) your participation in two, 5-day experimental trials that will be separated by 1 menstrual cycle which will involve eating a reduced-calorie diet (that we will provide) and completing two running programs, one with jumping exercises and one without. The screening visits will include taking your blood, performing tests of your aerobic capacity and exercise efficiency, and estimating your body composition. You will also record your food intake for 3 days and wear a smart watch (provided by the research team) for 3 days before the study begins and for 10 days during the experimental phases of the study. Additionally, you will wear a continuous glucose monitor and record the time of all meals and snacks during the 5-day experimental phases. Your blood will be drawn 5 times throughout the study (once at baseline and before and after each experimental phase). These tests are summarized in the table on page 7. The actual time and frequency of your visits may depend on your schedule and available appointment times.

During your first screening visit, you will determine whether you would like to participate by reviewing and discussing with a member of the research team what is involved in the study and the risks and benefits of doing so. We will ask you to complete some questions about your health history and eating and exercise habits. We will then measure your height and weight and ask you complete a pregnancy test before we perform a Dual-Energy X-ray Absorptiometry (DXA) scan to estimate your body composition and bone density.

During your baseline laboratory visit, we will ask you to come to the lab in the morning after not eating since 10PM the night before. At the Human Integrative Physiology lab, we will collect blood samples. At the Nutrition and Exercise Metabolism lab, we will have you perform a 12-20 min exercise test on the treadmill to measure your maximal aerobic capacity (Vo2max) during running. This visit collectively will take approximately 1 hour to complete.

3-days of usual diet and exercise. After completing the baseline testing visit, you will record the foods you usually eat over a 3-day period (2 days during the week, 1 day on the weekend). We will also ask you to complete a vitamin D questionnaire and wear a physical activity monitor to track the calories you burn each day.

Two 5-day experimental phases. The two, 5-day experimental phases will take place over a 5-week period. You will be scheduled to come in on the third or fourth day after you predict your monthly cycle will begin to start the 5-days of consecutive running. If your monthly cycle starts late or early, you will be asked to notify the research team and the date for your first run will be shifted. You will also be scheduled to come in the day

before each experimental phase (day 2 or 3 of your monthly cycle) to for a blood draw and to have a continuous glucose monitor placed on your arm. For this visit, we will ask you to come to the lab the next morning after not eating since 10PM the night before. If there is a conflict with your or the study staffs' schedule, blood will be drawn on the following morning. The day after your blood draw is complete, you will begin the first 5-day experimental phase. This will involve coming into the laboratory on 5 consecutive days to complete endurance training sessions on the treadmill under the guidance of a researcher who is also a certified trainer. In one phase, you will run on the treadmill for approximately 50-65 minutes depending on your running efficiency. In the other phase, you will complete 5 sets of 10 jumping exercises in the morning, and then return in the afternoon or early evening to run on the treadmill at a moderate pace for 50-65 minutes. You will also be provided with reduced-calorie meals and snacks to consume daily for the 5-day period and asked to record the time you eat each food on a mealtime log. We will ask that you a) not engage in any additional exercise, b) consume any additional foods or beverages (other than water and non-calorie beverages) outside of the ones provided during the 5-day programs, c) consume your meals and snacks around the same time each day, d) record the time you consume all foods and beverages during the experimental phases, and e) return all food packaging and uneaten food to the research team. Once you have completed the 5-day experimental phase, you will come to the lab the next morning after not eating since 10PM the night before to have a blood sample collected and the continuous glucose monitor removed.

After you complete the first experimental phase and have your blood sample collected on the following morning, you will resume your normal diet and exercise training until the start of your next menstrual period (approximately 3 weeks). The second experimental phase will look almost identical to the first phase. You will be asked to come into the lab on the morning after the first or second day of your menstrual period to have a blood sample collected and the continuous glucose monitor device placed. The following day, you will start the second 5-day experimental phase involving the consumption of a reduced-calorie diet and daily treadmill running with or without the jumping exercises (depending on whether you had them in the first phase or not). Once you complete the second phase, you will come into the lab the following morning to have a final blood sample collected and the continuous glucose monitor removed.

Please see "Overview of Testing Procedures" on page 7 for an outline of what will be completed throughout the study.

Is there any way being in this study could be bad for me?

There are some small risks to participating in the study resulting from reduced calorie intake that include loss of body weight, loss of muscle mass, increased hunger, mood disturbances and suboptimal micronutrient intake. Other small risks include sore muscles from the endurance training program, fainting or getting a small infection from giving blood samples, receiving a small amount of radiation from the DXA scans, or feeling extremely tired after the exercise tests.

> More detailed information about the risks of this study can be found under **"Is there any way being in this study could be bad for me? (Detailed Risks)".**

Will being in this study help me in any way?

We cannot promise any health or performance benefits to you or others from your taking part in this research. However, possible benefits may include receiving information about your body composition, bone density, aerobic fitness, and blood test. Please note, you should not consider your participation as a wellness or medical exam, and there will be no direct medical benefit to you. You should discuss any concerns about your health information with your health care provider.

Will I be paid for participating?

You will be compensated a total of $100 for completing this study. You will be compensated $25 for completing both the baseline visit and the first one-week diet and exercise phase. You will be compensated an additional $75 for completing the second diet and exercise phases. Cash payments will be provided after you complete the last day of each experimental phase.

What happens if I do not want to be in this research?

Participation in this research is completely up to you. You can decide to participate or not to participate.

If you are a student or employee, the decision whether to participate or not will have no effect on your grades, your employment, or your relationship with Virginia Tech.

Detailed Information: The following is more detailed information about this study in addition to the information listed above.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, contact Enette Larson-Meyer, the Principal Investigator of the study, at enette@vt.edu or 540-231-1025.

This research has been reviewed and approved by the Virginia Tech Institutional Review Board (IRB). You may communicate with them at 540-231-3732 or irb@vt.edu if:

- You have questions about your rights as a research subject
- Your questions, concerns, or complaints are not being answered by the research team
- You cannot reach the research team
- You want to talk to someone besides the research team to provide feedback about this research

How many people will be studied?

We plan to include about 20 women in this research study.

What happens if I say "Yes, I want to be in this research"?

If you say yes to participating, you will be enrolled in a 5-week endurance running study that will involve two, 5-day endurance training phases separated by approximately 3 weeks of your normal diet and exercise. During the 5-day endurance training phases, reduced-calorie meals and snacks will be provided to you and you will be asked to come into the lab on daily to perform a treadmill run for 5 consecutive days with jumping exercises and 5 consecutive days without jumping. The running phase you start with will be chosen by chance, like flipping a coin. Neither you nor the study team will choose which phase you start with. You will have an equal chance of starting with either phase.

In addition, you can expect that the following will take at each stage of the study. Also, please refer to "Overview of Testing Procedures" on page 7 which further outlines details of the study.

Baseline Screening Visit (approximately 1 hour)

Informed Consent: You will be provided an informed consent form by email or mail following the phone screening and before coming to the laboratory. You will review this form in-person with one of the researchers and have a chance to ask questions. You will need to sign this form before you can take part in the research.

Health History: You will be asked to complete a health and medical history questionnaire. This questionnaire is used to screen for health problems or reasons you should not participate in this study. If you have existing medical conditions that prohibit you from completing the exercise protocol, a history of menstrual disturbances, or are currently pregnant or lactating, you are not eligible to participate.

Energy Status: You will be asked to complete a questionnaire that assess risk of undereating in female athletes. This questionnaire is used to screen for risk of undereating, medication use, menstrual function, and injury history. If your score indicates "high risk", you will not be eligible to participate.

Urine Sample and Pregnancy Test: You will be asked to provide a small amount of urine before the DXA scan. This will be used to evaluate how hydrated you are. Additionally, you will be required to have a pregnancy test before the DXA scan. The urine sample will be used to perform this pregnancy test. If you are pregnant or the test indicates that you are pregnant, you will not be able to participate in this study.

Body Weight and Composition: Your height and weight will be measured on a digital scale. You will then lie on a hospital-type bed and a small amount of X-ray will be passed through your body to determine the amount of bone, muscle, and fat in your body. This unit is called a DXA scan. This test takes approximately 30 minutes which will involve a whole-body scan and two shorter scans of the hip and lower back (lumbar spine). There is no pain associated with the procedure.

Baseline Laboratory Testing Session (approximately 1 hour)

Blood Draw: Blood will be drawn at 5 timepoints throughout the study. The first blood draw will be taken at the baseline laboratory visit. Blood will be taken from a vein in your arm to measure hormones that reflect health status. These include a CBC (complete blood count) and thyroid-stimulating hormone. Samples of your blood (serum and plasma) will also be kept (banked) for a maximum of 10 years for possible measurement of other related factors that are not known to the researchers that may be important for bone health or general health outcomes**.**

Aerobic Capacity: You will begin running at a self-selected warm-up pace for 5 minutes. After the warm-up phase, the speed or grade of the treadmill will be increased every minute until you are too tired to continue. This will require that you wear a special face mask and nose clip to measure the air you are breathing in and out during the test. The full test will last for 12-20 minutes. After the test, we will offer you water, juice and/or a snack**.**

Normal Diet and Exercise (3 days)

Dietary Record: You will be asked to record all the food and beverages you have each day for 3 days in a row. We will ask you to record your food and beverages on 2 days during the week and 1 day on the weekend. You will also be asked to complete a vitamin D questionnaire with questions related to your food intake and sun exposure.

Physical Activity: You will be asked to wear a smart watch that will be provided by the research team during the 3 days that you complete your food record. ***You will arrange a time to drop off the smart watch and dietary records before the endurance training study begins*.**

Experimental Phases

Blood Draw: The remaining 4 blood draws will be taken during the experimental phases. Blood will be taken from a vein in your arm on days 1 and 7 of each phase (see table on page 7) to measure hormones and particles in the blood that are involved with bone health. These include progesterone, N-terminal propeptide of type 1 procollagen, undercarboxylated osteocalcin, sclerostin, C-terminal telopeptide of type 1 collage, parathyroid hormone, estradiol, insulin-like growth factor, hepcidin, insulin, markers of iron status, and thyroid hormones. Samples of your blood (serum and plasma) will also be kept (banked) for a maximum of 10 years for possible measurement of other related factors that are not known to the researchers that may be important for bone health**.**

Physical Activity and Continuous Glucose Monitoring: You will be asked to wear a smart watch that will be provided by the research team as well as a device called a continuous glucose monitor (CGM) during the time that you complete the running and reduced-calorie diet phases. The placement of the CGM device will require a sensor being inserted into the back of your upper arm. When the sensor is placed by the researcher, you can expect a sensation similar to a needle insertion for a blood draw. You may experience some discomfort during the insertion. This will take place under aseptic conditions; however, some pain, inflammation, redness/rash, swelling, minor bleeding and/or minor infection at the site is possible. This will be minimized by having a trained individual perform the procedure. You may also experience these symptoms as a result of contact between the adhesive pad of the sensor and your skin; allergic reactions can develop in response to the adhesive used to keep the device in place. If any of these symptoms occur, you have the ability to remove the CGM if you so desire, and the symptoms will clear up within a short period of time. You will wear the smart watch and the CGM for 7 days during the experimental phases.

Pregnancy test: You will be asked to complete a pregnancy test before starting the second experimental phase (Day 1 of the second reduced-calorie diet and running phase).

Mealtime Log: You will be asked to record the time you consume all meals, snacks, and beverages throughout the 5-day experimental phases. We will ask that you return all uneaten food and food packaging to the research team.

Table of Tests and Procedures at Baseline and During the Two Experimental Phases

Tests and Procedures			**Reduced-Calorie Diet and Running Phases (x2)**					
	Baseline	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
			Controlled Running with/without Jumping					
Body weight	x	x	x	x	x	x	x	x
Body composition and bone mineral density by DXA. *Note: a pregnancy test by urine is required before DXA*	x							
Baseline Screening Questionnaires	x							
Continuous glucose monitoring			x	x	x	x	x	
Blood								
CBC and TSH	x							
Progesterone and vitamin D		x						
Hormones and markers of bone health (N-terminal propeptide of type 1 procollagen,		x						x

undercarboxylated osteocalcin, sclerostin, C-terminal telopeptide of type 1 collagen, parathyroid hormone, estradiol, insulin-like growth factor, hepcidin, ferritin, iron, leptin, cortisol, nesfatin-1, insulin, thyroid hormones)								
Nutrition and Exercise Tracking								
Food records 3-day	x							
Vitamin D questionnaire	x							
Mealtime Log			x	x	x	x	x	
Exercise Tracking (smart watch)	x		x	x	x	x	x	
Fitness Testing								
Aerobic fitness (VO_{2max})	x							
Running efficiency			x				x	
Treadmill running			x	x	x	x	x	

Note: The experimental phases where you will consume a low-calorie diet and run on a treadmill will be completed on days 2-6 and repeated one time for a total of 10 days throughout the study.

What are my responsibilities if I take part in this research?

If you take part in this research, you will be responsible to:

- Give an accurate history of any health problems that you have or had or medicine that you take before the study begins.
- Tell the investigators of any discomfort or unusual feelings before, during, or after any of the study sessions.
- Be on time and attend all scheduled visits, including endurance training sessions.
- Follow all participant instructions for each endurance training session.
- Eat only the provided meals and snacks and only drink water or non-calorie beverages (e.g., black coffee, unsweetened tea).
- Refrain from all physical activity outside of the supervised exercise sessions. This includes additional running, lifting weights, exercise classes, high-intensity interval training, hiking, going for a walk for leisure, or any other physical activity or exercise. Activities such as getting dressed, walking to your car, bathing, cooking, etc. are permitted.

What happens if I say yes, but I change my mind later?

You can leave the research at any time, for any reason, without penalty.

Is there any way being in this study could be bad for me? (Detailed risks)

DXA Scan: You will be exposed to a small amount of radiation as part of the body composition testing during the DXA scan. The amount of radiation that you will receive in the DXA scans is far less than the amount that the Food and Drug Administration (FDA) allows per year. The amount you will receive from each scan is equal to $1/20^{th}$ of a chest X-ray. The more radiation you receive over your lifetime, the more your risk increases in

developing certain kinds of cancer. The radiation in this study is not expected to greatly increase these risks. The exact increase in this risk is not known.

Blood Draws: During each blood draw, you may experience slight discomfort. When the needle initially enters your skin, there will be a small pinch and it may hurt for a short time. The risks of having your blood drawn include development of a small bruise, development of a blood clot or infection in the vein, fainting, and/or dizziness. 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 your blood.

Exercise Efficiency and Aerobic Capacity: There is a small risk of injury (e.g., sprained ankle), complications requiring you to go to the hospital, 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 individuals. Only experienced staff members will conduct these tests and you will be monitored throughout the test for signs of problems. You will be tired after this test and may have sore muscles for a few days.

Low-Calorie Diets: Potential risks associated with consuming a low-calorie diet include weight loss, loss of muscle mass, mood disturbances, and low intake of vitamins and minerals. These risks will be minimized by providing a daily multivitamin to be taken on the 10 days when a low-calorie diet will be consumed. Meals and snacks will also be designed so that 20% of total calories come from protein to help protect muscle mass. It will be encouraged to eat the provided carbohydrate-based snack before the endurance training session each day to reduce feeling of fatigue or lightheadedness. Significant weight loss, decreases in muscle mass, and micronutrient deficiency are unlikely given the short-duration of this study.

Endurance Training: Risks associated with running on the treadmill include falls, stepping off of the belt, and post-exercise movement illusion (treadmill buzz). With any physical activity, there is a chance of muscle injury, ligament and tendon injury, as well as skeletal injury. Abnormal increases or decreases in blood pressure or cardiac arrhythmia are also possible but unlikely in young, healthy individuals with a background in endurance training. You are encouraged to let the researchers know if you develop any discomfort during or as the result of your treadmill runs, including excessive joint or muscle soreness, injury, or extreme fatigue.

High-Impact Jumping Exercises: Potential risks associated with high-impact jumping exercises include muscle injury, ligament and tendon injury, and skeletal injury. A low volume of jumps will be performed in this study (5 sets of 10 jumps per day) with a 60 second rest between each set of 10 jumps to reduce risk of excess strain on joints and bones. Jumps will be performed on a platform that measures force. Additionally, all jumps will be supervised by a NSCA-certified strength and conditioning specialist who will monitor movements for proper form and safety techniques. You are encouraged to let the researchers know if you develop any discomfort during or as the result of your jumping exercises, including excessive joint or muscle soreness or injury.

Continuous Glucose Monitoring: The placement of this device will require that a sensor is inserted into the back of your upper arm. Placement of the sensor may cause some pain during the insertion or lead to 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 that you 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, you have the ability to remove the CGM and these issues will clear up within a short time period.

What happens to the information collected for the research?

We will make every effort to limit the use and disclosure of your personal information, including study data and medical history data, only to people who have a need to review this information. We cannot promise complete confidentiality. Organizations that may inspect and copy your information include the IRB, Human Research Protection Program, and other authorized representatives of Virginia Tech. If identifiers are removed from your private information or from samples collected as part of this research, that de-identified information or those de-identified samples could be used for future research studies or distributed to another investigator for future research studies without your additional informed consent.

We may publish the results of this research, but we will keep private your name and any information that could identify you. We protect your information from disclosure to others as the law requires. We cannot promise complete secrecy.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Data or specimens collected in this research might have your identifying information removed and used for future research or given to another researcher for future research without your consent.

Can I be removed from the research without my OK?

The person in charge of the research study or the sponsor can remove you from the research study without your approval. Possible reasons for removal include:

- It is in your best interest
- If the researchers are unable to obtain measurements that are necessary for the study
- You develop an injury that does not allow you to fully participate in the study
- You become pregnant
- You are unable or unwilling to consume only the provided meals and snacks during the experimental phases
- You are unable to show up for the endurance training sessions or are unable to keep other scheduled appointments

What else do I need to know?

We will tell you about any new information that might affect your health, welfare, or choice to stay in the research.

If you become injured or sick from the research study that you are participating in, you will be referred to a clinic or to your personal physician or health care provider. Generally, this care will be billed to you, your insurance, or other third party. Virginia Tech has no program to pay for medical care for research-related injuries.

We will offer to share your individual test results with you. You may accept or decline these results.

Signature Block for Capable Adult

Your signature documents your permission to take part in this research. We will provide you with a signed copy of this form for your records.

Signature of subject	Date
Printed name of subject	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	

Appendix C: Study 1 Recruitment Materials

Volunteers Needed for Research Study on Bone Health in Female Runners

Female volunteers are needed for a study evaluating the effect of jumping exercises on bone health in long-distance runners eating a reduced-calorie diet.

IRB #22-168

You may be able to participate if you:

- Are a woman in good general health
- Are 18-35 years old
- Are a non-smoker
- Have a BMI between 18.5-25.0 kg/m2
- Have a regular menstrual cycle and are not on birth control (e.g., "the pill", IUD, and other birth control medications)
- Can run on 5 consecutive days for at least 60 minutes each day
- Not taking medications that could affect study results
- Not pregnant or lactating
- Are willing to complete supervised treadmill runs on 5 consecutive days on two occasions
- Are willing to eat a reduced-calorie diet that will be provided by the study team

You will receive information on your body composition, bone density, VO2max, running efficiency and $100 for your time.

This study will require visiting the lab on 17 days, 5 blood draws, consumption of meals provided by the research team, and participation in two phases of supervised treadmill runs and jumping exercises on 5 consecutive days in Blacksburg, VA.
Measurements will include body composition, bone density, physical activity, aerobic capacity, exercise efficiency, and dietary intake. You will also be asked to complete health screening questionnaires and wear a continuous glucose monitor.

VIRGINIA TECH

FOR MORE INFORMATION
Email VTNEMLab@gmail.com

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

Volunteers are needed for a study to examine the effect of jumping exercise on bone health in long-distance female runners eating a reduced-calorie diet.

You may be able to participate if you meet the following criteria:

- Woman in good general health
- Age 18-35 years old
- Not on birth control (including oral contraceptives, injections, or IUD)
- Has a regular menstrual cycle
- BMI between 18.5-25.0 kg/m2
- Can run on 5 consecutive days for 60-90 minutes each day
- Non-smoker
- Not pregnant or lactating

This study involves completing supervised treadmill runs on 5 consecutive days on two occasions. During one of those occasions, you will also be asked to perform jumping exercises. The two occasions will be separated by 1 menstrual cycle (approximately 3 weeks). Reduced-calorie meals and snacks will be provided during both 5-day periods.

Study measurements will include 5 fasted blood draws, body composition, bone density, VO2max, and exercise efficiency. Total time commitment will be 16 non-consecutive days of lab visits.

Participants will receive information on body composition, bone density, VO2max, running efficiency, and $100 for your time.

Please contact Trisha Sterringer (VTNEMLab@gmail.com) for more information.

Email To Send to Interested and Qualified Participants *after* the Phone Screening

Subject Line: "Bone Health in Female Runners Consent for your Consideration (IRB #22-168)"

Dear Participant [Insert Name],

Thank you again for your time to complete the phone screen for our study *The Effect of Impact-Loading on Bone Biomarkers in Energy-Restricted Females Runners (IRB #22-168)*. As promised, I am attaching a copy of the consent for the study for your careful consideration. The consent form provides a general overview of why we are doing the study, what we expect to get out of it, what you would do if you were to decide to participate and the potential risks to participating.
This copy is for your review only. You do not need to print it. If you decide to participate in the study, we will provide a hard copy for you to sign at the beginning of your screening visit which [*Insert A or B here] [(A) is scheduled at XX time on X date] or [(B) you can schedule when you are ready by replying to this email].
During your scheduled screening visit, we have set aside time at the start to discuss the consent and address any questions or concerns you have. You may also email questions before your scheduled visit by replying to this email.
Again, thank you for your interest in the study. We look forward to [(A) seeing you] or [(B) hearing from you].

Sincerely,
Trisha Sterringer, MS, RD, CSCS

Appendix D: Study 1 Questionnaires

HEALTH HISTORY SCREENING QUESTIONNAIRE

Please fill out as completely and accurately as possible.

Date _____/_____/_____

Name:_____ Ethnicity:_____

Address:_____City:_____State:_____Zip:_____

Age:_____ Phone #:_____ Alt #:_____

Email:_____@_____

CARDIOVASCULAR HEALTH HISTORY

Have you ever been diagnosed with or had any of the following?

Heart Attack?	Yes	No
Heart Surgery ?	Yes	No
Cerebrovascular accident?	Yes	No
Transient Ischemic Attack (TIA)?	Yes	No
Carotid Artery Disease?	Yes	No
Cardiac Catheterization?	Yes	No
Coronary Angioplasty?	Yes	No
Pacemaker/Implantable Cardiac Device?	Yes	No
Irregular Heart Rate/Heart Rhythm Disturbance?	Yes	No
Atrial Fibrillation?	Yes	No
Heart Valve Disease?	Yes	No
Heart Failure?	Yes	No
Heart Murmur?	Yes	No
Heart Transplantation?	Yes	No
Congenital Heart Disease?	Yes	No

Have you ever experienced any of the following symptoms:

Chest discomfort with exertion? Yes No

Unreasonable breathlessness? Yes No

Dizziness, fainting, or blackouts? Yes No

Syncope (loss of consciousness)? Yes No

Hypoxia (low oxygen levels)? Yes No

Do you currently take heart medications? Yes No

If yes, what?_____

Have you been diagnosed with diabetes (Type 1 or Type 2) or problems with blood sugar levels? Yes No

If yes, please note Type 1 or Type 2_____

If you circled yes to any of the above statements in this section, consult your physician or appropriate healthcare provider before engaging in exercise. You may need to use a facility with a* ***medically qualified staff.*

CARDIOVASCULAR RISK FACTORS

Are you a male over 45 years old? Yes No

Are you a female over 55 years old? Yes No

Have you had a hysterectomy with or without ovary removal? Yes No

Are you postmenopausal? Yes No

Do you currently smoke or have you quit within the last six months? Yes No

Is your blood pressure greater than 140/90 mm Hg? Yes No

I Don't Know

If known, what is your blood pressure? _____/_____ mm Hg

Do you currently take blood pressure medications? Yes No

Do you currently take any medications for your heart? Yes No

Is your blood cholesterol level greater than 200 mg/dl? Yes No

I Don't Know

Do you know your cholesterol or triglyceride levels? Yes No

If yes, Total Cholesterol _____ HDL _____

LDL _____ Triglycerides_____

Do you have a close blood relative who has suffered a heart attack or had any kind of heart surgery before the age of 55 (for father or brother) or age 65 (for mother or sister)? Yes No

Are you more than 20 pounds overweight? Yes No

I Don't Know

Are you physically inactive (i.e., do you get less than 30 minutes of physical activity less than three times a week)? Yes No

Have you had a recent surgery (in the past 2 years)? Yes No

If yes, please explain_____

_____

Have you had an exercise stress test, cardiac catherization, or echocardiogram? Yes No

If yes, please explain_____

_____

If you circled yes to two or more of the statements in the above section you should consult your physician or other healthcare provider before engaging in exercise. You might benefit from using a facility with a **professionally qualified exercise program.*

To the best of our knowledge, do you have any of the above fore-mentioned risk factors? Yes No**

*** You should be able to exercise safely without consulting your physician or other healthcare provider in a self-guided program or almost any facility that meets your exercise program needs.*

To the best of my knowledge, the information I have provided above is an accurate assessment of my health and medical history.

Name of Participant	**Participant's Signature**	**Date**
Name of Administering Staff	**Signature of Staff Member**	**Date**

Please stop here. The remainder of this Health History Screening Questionnaire will be administered to you by one of our staff.

Staff: Please administer the remaining portion of the Health History Survey.

GENERAL MEDICAL HISTORY

(this portion of the questionnaire is completed by oral interview of the participant by study staff)

Provide the following information:

Height:_____ Weight:_____ BMI (calculated):_____

Hip circ.:_____ Waist circ.:_____

Circle One

Do you drink alcohol? Yes No

If yes, how many drinks per week?_____

Are you taking any prescription or over-the-counter medication? Yes No

If yes, what medication and what dosage?_____

_____

Do you take any vitamins, supplements, performance enhancers or herbal/homeopathic medications?
Yes No

If yes, what type and what dosage?_____

_____

Have you been on a recent diet or a specific diet prescribed by a healthcare provider? Yes No

If yes, please explain_____

Have you been on a recent self-prescribed diet or a specific eating plan?
Yes No

If yes, please explain_____

For how long? _____

Have you been diagnosed with asthma, exercise-induced asthma, reactive airway disease, chronic obstructive pulmonary disease, or any other respiratory disease?
Yes No

If yes, please describe:_____

_____

Have you ever been diagnosed with cancer? Yes No

If yes, please describe when and what type:_____

_____

Have you ever undergone a lymphectomy? Yes No

If yes, please describe when and why?_____

Do you have musculoskeletal problems that limit your physical activity such as walking? Yes No

Do you have any bone, joint, ligament or tendon problems that bother you or cause discomfort on a regular basis? Examples include persistent knee or back pain. If yes, please describe _____ Yes No

_____

_____

Do you have concerns about your safety when you exercise or exert yourself? Yes No

Have you ever experienced burning or cramping sensations in your lower legs when walking short distances? Yes No

Have you ever been told that you were anemic? Yes No

If yes, how long ago? _____

Do you have other (recent, previous, current?) health problems, illnesses, diseases, infections, surgeries, allergies or hospitalizations including an eating disorder? _____

_____

Do you have any other health problems, illnesses, diseases, infections, surgeries, allergies, hospitalizations? Yes No

If yes, please explain_____

_____

Are you claustrophobic? Yes No
Do you have problems with having your blood drawn? Yes No
Do you have a fear of needles? Yes No
Do you have problems having your blood pressure taken in either arm? Yes No

FAMILY HISTORY

Please check all that apply

Family Member	High Blood Pressure	Diabetes Type I or II	Heart Disease	Obesity	Comments
Mother					If yes, was it before the age of 65? **Yes** **No**
Father					If yes, was it before the age of 65? **Yes** **No**
Sibling					Gender: Age:

Sibling					Gender: Age:
Paternal Grandmother					Age:
Paternal Grandfather					Age:
Maternal Grandmother					Age:
Maternal Grandfather					Age:

FOR FEMALES ONLY:

Are you pre-____, peri-____ or post-____ menopausal?
When was your last menstrual period? _____
How frequently do your menstrual periods come? _____

Are you using oral contraception (the Pill) or any form of estrogen only or combined hormonal contraception/ therapy, including creams, patches, or subdermal treatments? Yes No

If yes, what type?_____ Dosage?_____

If you are premenopausal:

Are you pregnant? Yes No I Don't Know

Could you be pregnant? Yes No I Don't Know

Are you trying to become pregnant? Yes No

If you are peri- or postmenopausal:

For how long?_____

Have you had a hysterectomy w/ or w/out ovary removal? Yes No

Are you currently taking any Hormone Replacement Therapy? Yes No

If yes, what type?_____How long?_____Dosage

Do you have any nutrition-related disorders that such as lactose intolerance, irritable bowel, food allerges, problems gaining weight, etc.

_____
_____

Administered by _____ (name of staff admistering form)

Name:_____ IRB #22-168

Screening for LOAD Study

Date:_____ **Screener:** _____**Study Code:**_____
Age:_____ **Email:**_____ **Phone:**_____
Reported: Height_____ in. Weight_____ lbs. BMI:_____ kg/m²

Consider yourself in good general health? **Yes** **No** **Not sure**

Pregnant or Lactating? **Yes** **No**

Currently trying to gain or lose body weight? **Yes** **No** **Not currently**
Reason _____
How long attempting? _____

<u>Endurance Training:</u>

1. How long have you been running long distance?

- ☐ Less than 1 year, _____ months or weeks
- ☐ 1-3 years
- ☐ 3-5 years
- ☐ 5-10 years
- ☐ 10 years+

2. On average, how many days a week do you currently run?

3. On average, how many miles do you currently run each week?

- ☐ Less than 20
- ☐ 20-30
- ☐ 30-40
- ☐ 40-50
- ☐ More than 50

If athlete doesn't fit above criteria, please explain:

4. On average, what is the distance of your normal training run (not including long runs)?
5. Do you currently engage in any cross-training (resistance training, swimming, cycling, etc.)? **Yes** **No**

If **yes**, how often?
6. Are you currently training for any races or athletic events **Yes** **No**

If **yes**,

Race date:
Race distance:

7. Do you have a heart or lung condition that would prevent you from participating in the study which will require daily exercise? **Yes** **No**

8. Are you currently dealing with any injury that would prevent you from running or engaging in your normal training? **Yes** **No**

If **yes**,

When the injury occurred:
Injury:

<u>Dietary Habits:</u>

Special diet/food preference: Vegetarian/vegan ____ Gluten-Free ____ Low-Carb ____

Paleo____ Other? _____

Do you have any food allergies or sensitivities? **Yes** **No**
If yes, which foods? _____
_____

<u>Medications & Supplements:</u>

Thyroid Hormone Replacement (e.g., Synthroid)	**Yes**	**No**
Anticonvulsants	**Yes**	**No**
Hormone Replacement	**Yes**	**No**
Birth Control	**Yes**	**No**

Dietary Supplements (Multi-vitamins, brand, dose, etc.):
_____
_____

Do you have normal menstrual cycles? **Yes** **No**

If yes, how frequently do they occur? _____
If yes, approximately how long do your menstrual cycles last? _____
If yes, when was your last period? _____

Have you ever been anemic? **Yes** **No**

If yes, when and any circumstances? _____

Have you ever had a blood or thyroid disorder? **Yes** **No**

If yes, when and any circumstances? _____

Have you ever been diagnosed with an eating disorder? **Yes** **No**

If yes, when and any circumstances? _____

Willing to participate in a supervised exercise program that would begin on the third or fourth day of your monthly cycle?	**Yes**	**No**
Willing to participate in supervised running program that will require you to run on 5 consecutive days in the NEM lab for approximately 60 minutes each day? (5 consecutive days on 2 occasions)	**Yes**	**No**
Willing to participate in supervised jumping program? (5 consecutive days on a randomly assigned occasion)	**Yes**	**No**
Willing to consume a reduced-calorie diet? (5 consecutive days on 2 occasions)	**Yes**	**No**
Comfortable with body composition testing?	**Yes**	**No**
Fear of needles or difficulty getting blood drawn?	**Yes**	**No**
Experience running on a treadmill	**Yes**	**No**

Appendix E: Study 1 Experimental Menus

Meal	Menu A	Menu B
Breakfast	• Low-sugar instant oatmeal • Nonfat plain Greek yogurt with blueberries and granola	• Whole wheat bagel with cream cheese • Nonfat plain Greek yogurt with blueberries and granola
Lunch	• Salad: Quinoa, lettuce, red cabbage, carrots, slivered almonds, sesame ginger dressing • Wheat crackers	• Salad: Brown rice, lettuce, cucumber, red pepper, feta cheese, low-fat Greek dressing • Wheat crackers
Pre-Run Snack *1-2 h before run*	• Pretzels (30g CHO)	• Pretzels (30g CHO)
Dinner	• Commercial frozen dinner with chicken • Whole wheat bread and butter • Dark chocolate square	• Commercial frozen dinner with beef • Whole wheat bread and butter • Dark chocolate square

Example of rotating menus for controlled diets for Study 1. *Menu A consumed on D1, D3, and D5. Menu B consumed on D2 and D4. Menus provided 30 kcal·kgFFM^{-1}·d^{-1} with 55% total kcal from carbohydrate (CHO), 20% from protein, and 25% from fat. Food items adjusted as necessary based on individual preferences to increase adherence.*

Smart Watch Set-up & Data Retrieval
Virginia Tech – Nutrition & Exercise Metabolism Lab

Providing the Smart Watch

1. Select smart watch and enter watch and wearing information on Garmin device tracking sheet Excel spreadsheet (found in Dropbox)
2. Sync watch with computer using USB cable and Garmin Express PC app
3. Log into Garmin Connect account associated with smart watch # - on website, with email and password on Excel spreadsheet under "Devices" tab
4. Click on picture of watch at the top and select "Device Settings"
5. Click on "Activity Tracking" tab
6. Enter Stride Length test results* - distance in feet and # of steps; Click on "Save Settings"
7. Click on "User settings" tab
8. Enter in info below; Click on "Save Settings"
    a. Birthdate
    b. Gender
    c. Height
    d. Weight
9. Click on "General" tab
10. Select Right or Left wrist; Click on "Save Settings"
11. Exit out of webpage and return to Garmin Express PC app
12. Sync the watch again to update settings and log back in to Garmin Connect to double-check settings

*Stride length test – Participant stands behind carpet line in the hallway, have participant walk 20 steps, place piece of tape at the back of the foot of the 20th step, and count the number of carpet squares (24" each) and measure the distance between the back of the piece of tape to the start of the carpet square and add together. Enter distance and # of steps into Activity Tracking on Garmin Connect website.

Retrieving the Smart Watch and Recorded Data

1. Collect smart watch, USB cable, and box from participant after the 3- or 5-day collection period
2. Connect smart watch to the computer using the USB cable
3. Open the Garmin Express app and allow smart watch to sync
4. When Garmin data file appears, copy the folder to the Smart Watch raw data folder on Dropbox
5. Click on option to load data and log into the Garmin Connect website account associated with the smart watch number
6. Verify that the data for the collection period looks correct and has uploaded to the account.
7. Contact Trisha Sterringer (tsterringer@vt.edu, 440-2215653) that someone has completed activity tracking
    a. Which participant
    b. Which watch
    c. What dates

Setting Up User Profile—STAFF ONLY

1. Hold button B down
2. Select ⚙
3. Choose “User Profile”
4. Select option to change

Setting Custom Stride Length

Garmin Connect Web

1. Connect device to computer with USB cable.
2. Sign into **Garmin Connect**
3. Select the device icon ⌚ (upper right corner)
4. Select your device from the list
5. Select **Activity Tracking**
6. Under Custom Stride Length, toggle **Walking** button to turn it on
7. Enter the known distance from your walk
8. Enter the number of steps it took to cover that distance
9. Scroll down and select **Save Settings**
10. Sync your device with Garmin Express or with the Garmin Connect app, if paired to a smartphone

Customizing Device

Activities and App Settings—STAFF ONLY

1. Hold button B
2. Select ⚙
3. Select “Activities and Apps”
4. Select chosen activity
5. Select “activity settings”
6. Definitions of settings that can be changed
    - Alerts: notifications during activity
    - Auto lap: creates a lap without pushing the button at a predetermined distance
    - Auto pause: stops recording when under a certain speed or stopped
    - Auto rest: stops recording during pool swim, creates rest interval
    - Auto scroll: flips through data screens during activity
    - Auto set: starts and stops strength sets during activity
    - Data screens: customize the data screens during activity
    - Edit weight: add weight to an exercise set during strength
    - GPS: sets mode for GPS antenna
    - Pool size: sets size of pool for pool swimming
    - Vibration alerts: notify to inhale or exhale during breathwork - NO VIBRATION FEEDBACK

Treadmill Runs
LOAD – Nutrition & Exercise Metabolism Laboratory

PRESCRIPTION RUN

Procedure:

1. Calibrate the metabolic cart after the heater has warmed up for at least 30 minutes.
2. Weigh participant without shoes.
3. Record the time of the participant's last meal/snack.
4. Place a heart rate monitor on the participant by first wetting the electrode strip and skin of the participant. The HR monitor should fit snuggly against the participant's skin without any gaps.
5. Place the facemask on the participant connected to the metabolic cart.
6. Start the participant's smartwatch for "treadmill run".
7. Begin the running economy test at a speed of 5.5mph and 0% grade. Increase pace by 0.5mph in 4-minute stages. Record the RPE during the last minute of each stage. Collect HR every 20 seconds during the last 2 minutes.
8. When the economy test is complete, proceed to estimate target pace.
9. Increase incline to 1% grade and set the speed to the target pace estimated using the ACSM equation. If the participant seems more efficient based on the economy test, increase the pace to a speed that seems close to the target 65-70% VO_2max. Each phase will be 4 minutes with the last 2 minutes of steady state data collected.
    a. **If VO_2 is reached:** Do not end the test until the 4-min stage is complete.
    b. **If VO_2 is exceeded:** Do not adjust the speed until the 4-min stage is complete. Then reduce the speed as necessary.
    c. **DO NOT stop the test or adjust the speed until the entire 4-min stage is complete**.
10. When the test is complete, remove facemask and save excel file. In the notes of the metabolic test, include the paces used for the economy test and prescription stages. Save file in the data folder on DropBox or OneDrive.
11. Provide a weighed water bottle. Instruct the participant to consume as much water as they would like during the remainder of the run. When the run is complete, reweight the bottle.
12. Record the time when the run begins and start a timer. Pause the timer any time the participant needs to use the restroom or take a break. Collect HR every 5 min.
13. Open the prescription excel file (*DropBox > LOAD Study > Study Forms > Data Collection Sheets > Prescription Spreadsheet Template*). Enter the last 2 minutes of data for the VO_2 and REE/min. The excel sheet will calculate the remaining duration of the run.
14. At the end of the run, remove HR monitor and stop smartwatch. Weigh the water bottle to calculate amount of water consumed.
15. Provide lunchbox.

RUN ON DAYS 2-4

Procedure:

1. Weigh participant without shoes.
2. Record the time of the participant's last meal/snack.
3. Provide water bottle with the same amount of water that was consumed on day 1. Instruct participant to consume the entire bottle during the run.
4. Place a heart rate monitor on the participant by first wetting the electrode strip and skin of the participant. The HR monitor should fit snuggly against the participant's skin without any gaps.
5. Start the participant's smartwatch for Treadmill Run.

6. Record the time when the run begins and start a timer. Pause the timer any time the participant needs to use the restroom or take a break.
7. Begin with a 5-minute warm-up at a self-selected pace of 5.5, 6.0, or 6.5 mph at 0% grade. After warm-up, increase speed to prescription pace and grade to 1%. Collect HR and RPE every 5 min.
8. At the end of the run, remove heart rate monitor and stop smartwatch.

DAY 5 RUN

Procedure:

1. Calibrate the metabolic cart after the heater has warmed up for at least 30 minutes.
2. Weigh participant without shoes.
3. Record the time of the participant's last meal/snack.
4. Provide water bottle with the same amount of water that was consumed on day 1. Instruct participant to consume the entire bottle during the run.
5. Place a heart rate monitor on the participant by first wetting the electrode strip and skin of the participant. The HR monitor should fit snuggly against the participant's skin without any gaps.
6. Place the facemask on the participant connected to the metabolic cart. Set the exercise testing to "submaximal" and then begin the test.
7. Start the participant's smartwatch for Treadmill Run.
8. Begin the running economy test at a speed of 5.5mph and 0% grade. Increase pace by 0.5mph in 4-minute stages.
9. When economy test is complete, remove facemask and save excel file (*Desktop > LOAD > Economy).*
    a. BEFORE SAVING: In the notes section on the economy test put the time the test ended and the speed of the 3 stages for the economy test.
        i. Example: "Test ended at 12:01. Economy stages 5.5, 6.0, 6.5 mph at 0% grade."
    b. Print a copy of the metabolic report.
    c. Save the excel file on the cart computer.
        i. Location: *Desktop > LOAD > Economy*
        ii. File Name: ParticipantID_Economy_Pre/Post_RUN/JUMP
    d. Save the excel file on DropBox or OneDrive
        i. Location: DropBox > LOAD study > Data > Economy > Participant ID
10. The participant can then start running again at the target pace. Be sure to record the time when the run begins and start a timer. Pause the timer any time the participant needs to use the restroom or take a break.
11. Calculate the remaining duration for the run using the steps below:
    a. Open the Prescription Spreadsheet Template on DropBox (*DropBox > LOAD Study > Study Forms > Data Collection Sheets > Prescription Spreadsheet Template*)
    b. the last 2 minutes of data for the VO_2 and the spreadsheet will automatically calculate the remaining duration for the run.
12. Collect HR every 5 min.
13. At the end of the run, remove heart rate monitor and stop smartwatch.

Blood Draw
LOAD – Nutrition & Exercise Metabolism Laboratory

Supplies

ALL BLOOD DRAW VISITS

2 Chuck pads
Nitrile gloves
Tourniquet
CVS – Closed venous system ("butterfly")
Vacutainer holder
2 Alcohol preps
Coban
Gauze pads

SCREENING

1 4mL purple top tube
1 8.5mL red/gray "tiger" top tube
Non-sterile urine cup
Pregnancy test
1 small biohazard bag (for Lab Corp)
Lab Corp sample requisition form

PRE/POST INTERVENTION

1 4mL purple top tube
2 8.5mL red/gray "tiger" top tube (1 for Lab Corp, 1 for storage)
2 small biohazard bags (for Lab Corp; one frozen one room temp)
2 Lab Corp sample requisition forms (one for frozen, one for room temp samples)
2 purple cryovials (for Lab Corp)
6 orange top cryovials (for storage)

ADDITIONAL FOR BEFORE SECOND INTERVENTION

Non-sterile urine cup
Pregnancy test

Blood Collection Procedure

SCREENING

1. Set up for blood draw.
2. Label 4mL purple and 8.5mL red/gray "tiger" top tubes with subject ID# (LOAD ##), DOB, collection time, & date.
3. Once fasting blood sample is collected, invert all tubes 6-8 times.
4. Allow red/gray "tiger" top tube to sit for at least 15 min at room temp. 4mL purple top tube rests on ice.
5. Centrifuge 4mL purple top and 8.5mL red/gray "tiger" top tubes after 15 min (gray top tubes should be clotted) at 2500 rpm for 15 min (or 3500 rpm for 13 min) at 4°C. Ensure centrifuge is balanced.
6. Store 8.5mL red/gray top (ensure stopper is between the two layers) and 4mL purple top tubes in small biohazard bag at room temp.

PRE/POST INTERVENTION

1. Set up for blood draw.

2. Label both 8.5mL red/gray “tiger” top tubes with subject ID# (LOAD ##), DOB, collection time, & date. Label purple cryovials (for Lab Corp) with subject ID# and timepoint, DOB, collection time and date, and “PTH frozen from EDTA” OR “INSULIN frozen from SST”. Label storage cryovials (orange top) with subject ID#, collection date, P/S, study timepoint (1 plasma, 5 serum).
3. Once fasting blood sample is collected, invert all tubes 6-8 times.
4. Allow red/gray top tubes to sit for at least 15 min at room temp. 4mL purple top tube rests on ice.
5. Centrifuge 4mL purple top and red/gray top tubes after 15 min (red/gray top tubes should be clotted) at 2500 rpm for 15 min (or 3500 rpm for 13 min) at 4°C. Ensure centrifuge is balanced.
6. Using transfer pipet, transfer ~1mL plasma (4mL purple top) into the purple cryovial labeled PTH for Lab Corp. Transfer the remaining plasma into the one storage cryovial (orange) labeled for plasma. Identify the red/gray “tiger” top with the greater volume of serum. This one will be used for Lab Corp. Using a new pipet, transfer ~1mL serum into the purple cryovial labeled INSULIN for Lab Corp. Reseal the red/gray “tiger” top tube for Lab Corp. Transfer serum from the second red/grey “tiger” top tube into 5 serum labeled cryovials. Place the 2 purple cryovials for Lab Corp into the -80 freezer. Store the 6 orange cryovials in the -80 freezer. Indicate the # of cryovials collected on the LOAD blood collection form as well as the storage box and slots (also on freezer blood sample tracking sheet).

LabCorp Requisition Form

1. Enter date specimen collected, mm/dd/yyyy
2. Enter time specimen collected using a 24-hour clock, hh:mm
3. Enter patient initials, LOAD
4. Enter patient number, study number
5. Enter sex, F
6. Enter patient date of birth, mm/dd/yyyy
7. Mark Fasting or Nonfasting at top of the page
8. Enter patient visit name, study timepoint (SCREEN, PRE/POST A or B)
9. “X”: Required tests based on timepoint
    a. SCREEN: CBC, TSH
    b. PRE/POST A and B
        i. Frozen: Insulin, PTH
        ii. Room Temp: Cortisol, Iron, Ferritin
10. Place order form in the side pouch of the blood sample biohazard bag.
11. For frozen samples: Wait at least 30 minutes for samples to freeze completely. Place frozen samples in blood sample biohazard bag and place inside of the Lab Corp frozen specimen samples box with an icepack on the top and bottom of the sample (small gel ice packs located in top shelf of standing freezer).

Courier Service

1. **Preferred: Sign in to LabCorp Link account to schedule pick-up online. or Call LabCorp at 540-563-9852, opt 3 ASAP to schedule a pick-up for account #45050060 at 1872 Pratt Dr., Suite 1575, Garvin Building.
2. At pick-up time or earlier, place biohazard bag into the collection box in the hall. Key to collection box in Elaina’s upper side desk drawer, labeled LabCorp.

A report will usually be ready the following day via LabCorp Link. Save results on Dropbox. Print results and place in subject’s study folder in Garvin.

If trouble with report, contact Suzanne Carlisle, carlis1@labcorp.com or Loretta Ashcraft, ashcral@labcorp.com.
Print and place lab report in participant subject folder.

Continuous Glucose Monitor
Virginia Tech – Nutrition & Exercise Metabolism Lab

Forms: CGM participant tracking sheet, CGM device tracking spreadsheet

Supplies

-Application:
CGM sensor
CGM reader
Chuck pad
Nitrile gloves
Alcohol wipe
Sharps container
Tegaderm tape, 2 patches

-Removal:
Gloves
Alcohol wipe
Small biohazard bag

Set-up

- Select CGM reader, sensor box
- Record serial # and participant information on CGM device tracking sheet
- Open sensor box and remove Sensor Pack, Sensor Applicator, and alcohol wipe
- Check that Sensor Pack and Applicator serial #'s match

Sensor application

- Select site on the back of the mid-upper arm. Determine if participant is a side sleeper and avoid the arm that is slept on regularly.
- Technician put on gloves
- Clean site with alcohol wipe, let dry.
- Take off lid to Sensor Pack and unscrew cap from Sensor Applicator
- Line up dark marks on Sensor Applicator and Sensor Pack. Press down firmly on Sensor Applicator until it comes to a stop.
- Lift Sensor Applicator out of Sensor Pack
- Place Sensor Applicator over site. Hold participant's arm and push down firmly until there is a "click" from the Sensor; remove Applicator.
- Apply Tegaderm tape, if needed. Provide extra Tegaderm tape, if necessary.
- Discard Applicator in Sharps biohazard container.
- Activate sensor by scanning with the reader*. Turn on reader by pressing the Home Button, select "+Start New Sensor", hold the reader within 1.5 inches of the sensor, listen for "beep". Wait 2 min, press "yes" to rescan the sensor, hold reader within 1.5 inches of the sensor; listen

for “beep”; press OK to go back to the Home Screen when reader has Sensor Working message.
- Clean reader with damp microfiber cloth before storing

Data collection

- Turn reader on; use the same one that started the data collection.
- Select “Get Sensor Data”
- Hold reader within 1.5 inches of the sensor; listen for “beep”

Sensor removal

- Loosen sensor adhesive (remove Tegaderm tape first, if worn) and slowly remove from skin.
- Place sensor in Sharps/biohazard container
- Clean participant’s arm with alcohol wipe

Data download

- Connect reader to computer using yellow USB cable
- Sign in to HNFE’s Clinical Studies LibreView.com account
- Select “Upload device” and select “Create Report Linked to Patient” for new participant
- For the first collection, create participant profile, includes first and last name as ID# and 01/01/birth year as DOB. All following testing, search for the specific participant.
- Download raw glucose data Excel spreadsheet on laptop/PC and back up on a thumb drive

Notes

- Provide participant with CGM tracking sheet and instructions
- Do not wear CGM during DXA
- Cannot collect multiple CGM sensor data on one reader. Once data is collected, it must be uploaded to a computer/LibreView account before another sensor is read.
- First reading should appear ~1 hour after sensor activation
- Review data for overnight sleep compression artifact (~ <50 mg/dL)

***Setting up the Reader**

- Press Home Button to turn on the reader.
- Select preferred language, if prompted, and press OK
- Set the current date and press next
- Set the current time and press next
- Set the target glucose range (most literature indicates 70-180 mg/dL); this can be changed in the software for participant reports. Press next.
- Press done to return to Home screen
- Turn on sound in settings, if needed