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The Effects of Energy Deficiency, Exercise and Dietary Protein on Skeletal Muscle and Bone

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To everyone you believed in me more than I believed in myself, thank you.

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Abstract

Research on energy status is historically conducted from either an energy balance perspective in literature on weight loss in overweight persons or an energy availability perspective in literature on athletic populations. Regardless of which perspective is used to quantify energy status, when energy intake is insufficient relative to energy expenditure, an energy deficiency results. Despite being required for weight loss, energy deficits carry a bevy of negative side effects, including notable losses of skeletal muscle and bone tissues, and can result in adverse events such as fractures. In some circumstances, such as during intentional weight loss, energy deficits are unavoidable and, as such, strategies which can minimize the negative side effects are invaluable to reduce the changes of an adverse event, like a fracture, occurring. The studies enclosed in this dissertation will take a closer look at two strategies with the potential to attenuate the negative effects on skeletal muscle and bone as a result of energy deficiency: consumption of a high-protein diet and resistance training.

In the first study, the potential for a high-protein diet to attenuate changes in systemic hormones like insulin-like growth factor 1 (IGF-1), composition of weight loss and markers of bone turnover in response to an energy deficit created by a combination of aerobic exercise and energy restriction was explored. In contrast to the hypotheses, the high-protein diet was unable to significantly affect any of the investigated outcomes impaired by the energy deficit. As a result, in the second study, protein supplementation was instead combined with a bout of resistance exercise to investigate (1) whether the anabolic response of IGF-1 to resistance exercise was compromised by the energy deficit as at rest and (2) whether post-exercise protein supplementation could improve this response. This study showed that the IGF-1 response to a bout of resistance exercise was impaired and post-exercise protein supplementation was insufficient to rescue this response. To follow up on this novel finding, the third study included a meta-analysis aimed to investigate whether this acute impairment of the hormonal response to resistance exercise in an energy deficit translated into impaired long-term adaptations to resistance training, namely lean mass and strength gains. The meta-analysis revealed that gains in lean mass, but not strength, were impaired by conducting resistance training in an energy deficit. In addition, the degree of energy deficit scaled linearly with the change in lean mass. Overall, this dissertation reinforces the consequences of energy deficiency for skeletal muscle and bone even in the presence of a high-protein diet or resistance training.

List of Included Scientific Papers

I. **Murphy C**, Bilek LD and Koehler K. “Low energy availability with and without a high-protein diet suppresses bone formation and increases bone resorption in men: a randomized controlled pilot study.” *Nutrients*, 2021; 13(3): 802. DOI: 10.3390/nu13030802.
Impact Factor: 4.546

II. **Murphy C** and Koehler K. “Caloric restriction induces anabolic resistance to resistance exercise.” *European Journal of Applied Physiology*, 2020; 120(5): 1155-1164. DOI: 10.1007/s00421-020-04354-0.
Impact Factor: 2.580

III. **Murphy C** and Koehler K. “Energy Deficiency Impairs Resistance Training Gains in Lean Mass but not Strength: A Meta-Analysis and Meta-Regression.” *Scandinavian Journal of Medicine and Science in Sports,* 2021; epub ahead of print. DOI: 10.1111/sms.14075.
Impact Factor: 3.255

1 Introduction

The following thesis describes the negative effects of energy deficiency and how exercise, particularly resistance exercise, and dietary protein can be used to attenuate them. In the first subsection (1.1), the two most common perspectives on energy status are introduced. Afterwards, the effects of energy deficiency are outlined (1.2) and the use of resistance exercise and dietary protein to protect against these effects will be discussed (1.3). The following subsection (1.4) presents the aims of the dissertation and in the final subsection (1.5), the methods used in this dissertation are presented.

1.1 Energy Status

Energy status can be calculated from two distinct perspectives, energy balance and energy availability, each represented graphically in Figure 1. Energy balance subtracts energy intake from the sum of all components of energy expenditure and is typically expressed in kilocalories (kcal). In this way, energy balance can be viewed as an output measure of what remains after all energy components are considered. If energy intake and energy expenditure are equal, energy balance is achieved. When energy intake exceeds energy expenditure, a positive energy balance or an energy surplus occurs. Conversely, when energy expenditure exceeds energy intake, a negative energy balance or an energy deficit occurs. Quantification of energy status from an energy balance perspective parallels weight status such that an energy surplus leads to weight gain and an energy deficit leads to weight loss. On the other hand, energy availability is calculated by subtracting exercise energy expenditure from energy intake and is expressed in terms of kcal · kg fat-free mass^{-1}. In contrast to energy balance, energy availability is viewed as an input measure of energy available to maintain physiological function once the cost of exercise is accounted for. In order to maintain optimal physiological functionality, an energy availability of 40 to 45 kcal · kg fat-free mass^{-1} is required. Landmark literature in energy availability has defined a threshold of 30 kcal · kg fat-free mass^{-1} at or below which weight loss is accompanied by a broad spectrum of physiological effects which will be discussed in the coming paragraphs (Loucks & Thuma, 2003).

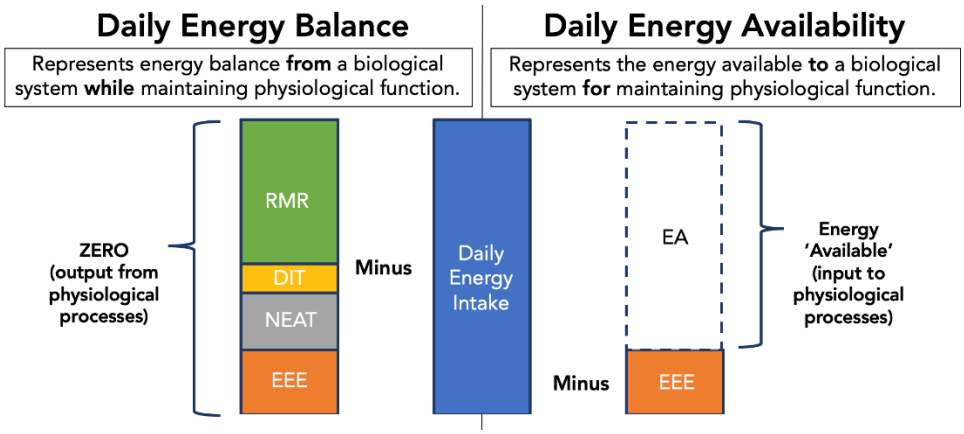

Figure 1. Illustration from Areta JL, Taylor HL and Koehler K (2021). Depiction of an individual's energy status from the two distinct perspectives of energy balance and energy availability. RMR, resting metabolic rate; DIT, diet-induced thermogenesis; NEAT, non-exercise activity thermogenesis; EEE, exercise energy expenditure; EA, energy availability.

Energy status is important because it underscores weight status. According to the World Health Organization, more than 50% of adults worldwide are overweight or obese (World Health Organization, 2020). Understanding how to measure and manipulate energy status is a crucial tool for combating the obesity epidemic, as energy status has been shown to be the primary driver for obesity risk (Howell & Kones, 2017). However, energy status also has implications beyond inducing weight loss in overweight and obese populations. Though the energy balance perspective is the primary focus of obesity research, another perspective, that of energy availability, has become increasingly popular over the past couple decades thanks in large part to growing interest in the female athlete triad, a framework describing the combination of low energy availability, low bone mineral density and menstrual disturbances found in exercising women (De Souza et al., 2014). However, the combination of low energy availability, low bone mineral density and reproductive impairments may also occur in men and, in both sexes, occurs alongside a bevy of additional health consequences. These expansions in understanding informed the development of the relative energy deficiency in sport, or RED-S, framework depicted in Figure 2 (Mountjoy et al., 2018). In both frameworks, insufficient energy status, labelled either as low energy availability, an obvious derivative of the energy availability perspective, or energy deficiency, a more neutral term with regards to energy status perspective, is the underlying element responsible for the other aspects of the frameworks.

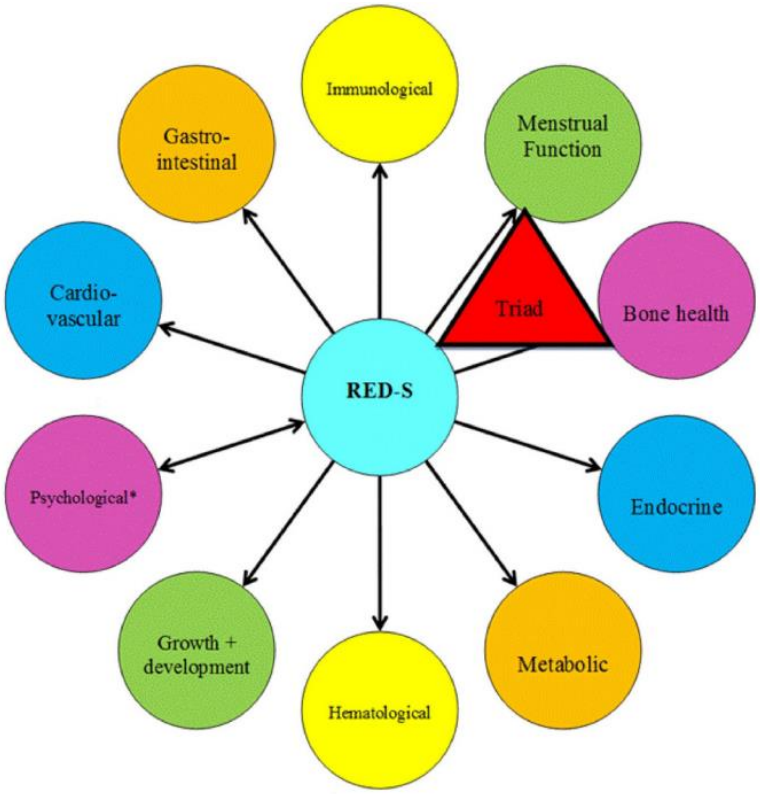

*Figure 2. Illustration from Mountjoy M et al. (2018). Illustration of the many health facets addressed by the Relative Energy Deficiency in Sport (RED-S) perspective compared to the Female Athlete Triad framework. * indicates an acknowledgement that "Psychological consequences can either precede RED-S or be the result of RED-S" as further indicated by the double-sided arrow.*

Throughout the following dissertation, the terms *energy deficiency* and *energy deficit* will be broadly used to generally discuss an insufficient energy status as some of the discussed literature has been written from an energy balance perspective while the rest was written from an energy availability perspective. Terminology specific to individual studies may deviate from this general guideline when specific quantifications or thresholds are presented or as is otherwise appropriate.

1.2 Effects of Energy Deficiency

Energy status has implications for a broad range of effects beyond determination of weight status. The hormonal, substrate and biomarker disturbances secondary to energy deficiency that underly most of the negative consequences associated with this state of insufficient energy

are summarized in Figure 3. These disturbances have a range of effects leading to reproductive disruptions and loss of bone mineral density, pillars of the aforementioned Female Athlete Triad and RED-S frameworks, as well as loss of lean mass. Throughout the following section, the underlying hormonal, substrate and biomarker disturbances as well as their consequences will be described in greater detail.

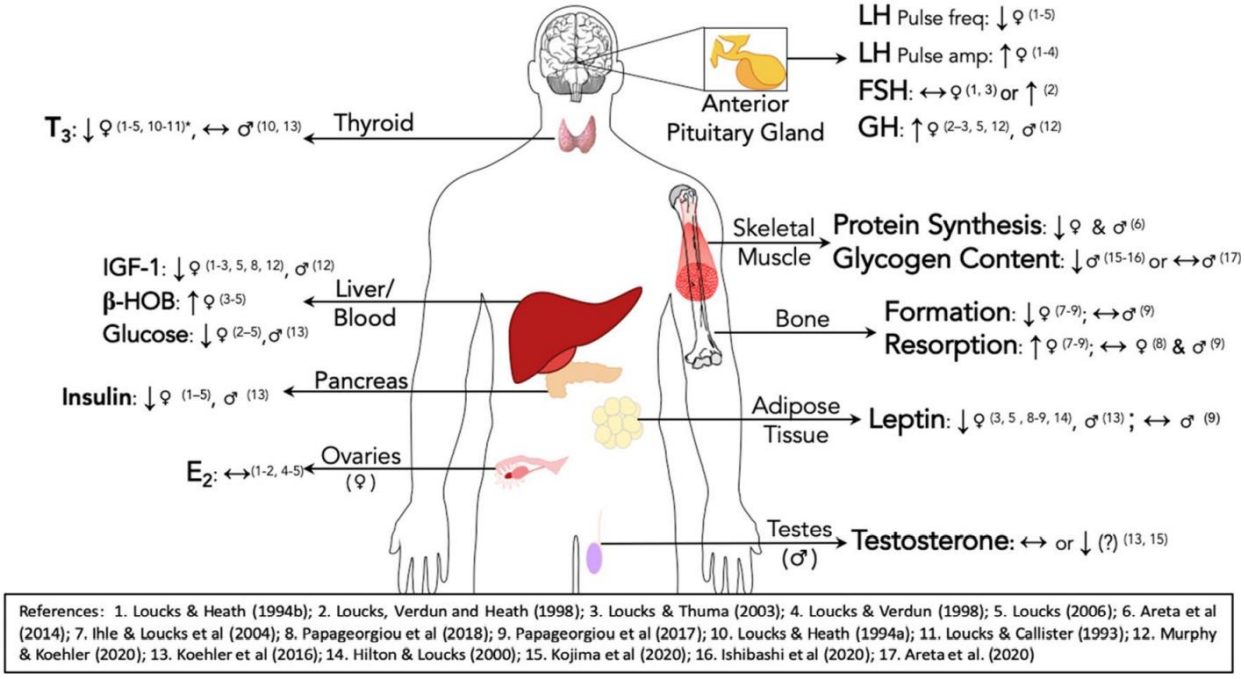

Figure 3. Illustration from Areta JL, Taylor HL and Koehler K (2021). Summary of the effects of energy deficiency on hormones, biomarkers and substrates in short-term interventions. B-HOB, beta hydroxybutyrate; E_2, estrogen; FSH, follicle stimulating hormone; GH, growth hormone; IGF-1, insulin-like growth factor 1; LH, luteinizing hormone; T_3, triiodothyronine.

Of the hormonal outcomes summarized above, the most significant of these in relation to the losses of bone and lean mass is the disruption of the growth hormone (GH):insulin-like growth factor 1 (IGF-1) axis as both GH and IGF-1 play significant roles in the development of the musculoskeletal system (Tritos & Klibanski, 2016). In energy-replete physiology, the hypothalamus produces GH releasing hormone which stimulates the production of GH in the anterior pituitary. GH secreted from the anterior pituitary then stimulates the production of IGF-1, primarily in the liver, which, in turn, inhibits the further production of GH releasing hormone and GH (Vottero, Guzzetti, & Loche, 2013). However, energy deficiency has been shown to disturb this relationship such that GH is increased but results in a counterintuitive decrease in

IGF-1 (Fazeli & Klibanski, 2014; Miller, 2011). This disruption scales with the size of the energy deficit (Loucks & Thuma, 2003) and reductions in IGF-1 secondary to energy deficiency have been associated with bone loss (Ihle & Loucks, 2004) as well as loss of bone mineral density (De Souza & Williams, 2005). Thus, preserving the sensitivity of the GH:IGF-1 axis during energy deficiency may provide one potential target for attenuating the loss of bone mineral density associated with energy deficiency.

Leptin is another metabolic hormone linked to bone health and reduced in a dose-dependent manner by increasing levels of energy deficiency (Loucks & Thuma, 2003). Leptin is secreted primarily from adipose tissue, but adipocytes within bone marrow also produce leptin and this proximity may partially explain the link between leptin and bone health (Hamrick & Ferrari, 2008; Reid, Baldock, & Cornish, 2018). While reductions in leptin have been viewed as positive outcomes in the context of intentional weight loss, low leptin levels have been associated with an increased incidence of fracture (Nakamura et al., 2020; Schett et al., 2004) and may both directly and indirectly contribute to the adverse relationship between energy deficiency and bone health (Upadhyay, Farr, & Mantzoros, 2015). Directly speaking, leptin binds to bone marrow-derived stromal cells and inhibits the differentiation of osteoclasts among other local effects (Hamrick & Ferrari, 2008). In addition, leptin has been shown to stimulate the GH:IGF-1 axis, which may indirectly contribute to leptin's bone formation-stimulating effects (Hamrick & Ferrari, 2008). Thus, reductions in leptin may lead to both an increase in bone resorption and a decrease in bone formation, two characteristics of an energy deficient state. This suggests that maintenance of leptin could provide another targeted outcome to aid in maintenance of bone health during periods of energy deficiency. However, it is important to note that though leptin is produced predominantly by adipose tissue, changes in leptin are visible long before changes in fat mass manifest (Inoue et al., 2018) and, thus, this does not suggest maintenance of fat mass during periods of energy deficiency is a desirable outcome.

The causal relationship between energy deficiency and reproductive hormones leading to menstrual disturbances is well-established in women (Nattiv et al., 2007) and this relationship has even been shown to be dose-dependent (Loucks & Thuma, 2003) similar to the aforementioned hormones. There is also preliminary evidence of compromises to reproductive function in men (Lane, Magallanes, & Hackney, 2019), though the body of literature is still lacking compared to that of their female counterparts. Study of and consensus on this matter is likely complicated by the large variation in basal levels and response to exercise of testosterone

(Cano Sokoloff, Misra, & Ackerman, 2016). While a complete discussion of the effects of energy deficiency on reproductive function is beyond the scope of this dissertation, hypogonadism resulting from energy deficiency directly contributes to the shifts in bone metabolism leading to bone loss (Misra, 2012). Estrogen decreases bone resorption by inhibiting osteoclast activity through reducing RANKL binding to RANK (Misra, 2012). Testosterone exerts both indirect effects via aromatase conversion to estrogen as well as direct effects both inhibiting bone resorption and increasing bone formation (Misra, 2012). These interwoven, synergistic relationships between the gonadal steroids, combined with the aforementioned contributions of the GH:IGF-1 axis and leptin, shows the interconnectedness of the hormonal responses to energy deficiency and its effects on bone health.

Compromised bone health is one of the longest-standing and well-established consequences of prolonged energy deficiency with an abundance of literature evidence behind this association. Cross-sectionally, female athletes with menstrual irregularities as a result of energy deficiency have been demonstrated to have lower bone mineral density and a higher incidence of fracture than their eumenorrheic contemporaries (Ackerman et al., 2015), and this association has also been reproduced prospectively across multiple cohorts of athletic women (Barrack et al., 2014; Tenforde et al., 2017). The relationship between bone health and energy deficiency is also visible in non-athletic, weight loss populations where the loss of 10% body weight has been shown to decrease bone mineral density by 5% in elderly men (Ensrud et al., 2018) and double fracture risk in women (Langlois et al., 2001). Engaging in weight loss early in life has also been shown to produce similar effects of reducing bone mineral density and increasing fracture risk later in life (Shen et al., 2020). Due to these negative associations and the fact that bone lost during weight loss is not easily restored (Villalon et al., 2011), the relationship between bone health and energy deficiency is typically measured in response to short-term interventions to evaluate the change in markers of bone metabolism long before changes in bone mineral density can occur. Short term energy deficits of <10 days have been shown to alter markers of bone metabolism in women (Ihle & Loucks, 2004), men (Zanker & Swaine, 2000) and a pooled sample of men and women (Papageorgiou et al., 2017). These alterations include decreases in markers of bone formation and increases in markers of bone resorption. Together, these outcomes indicate a shift towards bone catabolism as a result of energy deficiency. Young athletes with low bone mineral density increase their risk for fracture, the occurrence of which interferes with training and competition (Mountjoy et al., 2018). Furthermore, impaired accrual of peak bone mass in young athletes may also increase risk of osteoporosis later in life (Rizzoli,

Bianchi, Garabédian, McKay, & Moreno, 2010). Osteoporosis is associated with fractures which can lead to catabolic crises and accelerate the development in sarcopenia in older individuals (English & Paddon-Jones, 2010). Thus, regardless of the point in the lifespan at which energy deficiency occurs, there is a demonstrated link to adverse effects on bone health with both short-term and long-term consequences.

Unlike compromised bone health, reduced lean mass is not a pillar of the Female Athlete Triad nor RED-S frameworks. In older adults, the link between decreases in bone and lean masses are recognized through the association of osteoporosis and sarcopenia (Edwards, Dennison, Aihie Sayer, Fielding, & Cooper, 2015). In younger adults, this recognition is not as canonical, but the decreases in muscle protein synthesis (Areta et al., 2014) and increases in muscle protein breakdown (Carbone, Pasiakos, Vislocky, Anderson, & Rodriguez, 2014) observed in response to short-term energy deficits parallel the decreases in bone formation and increases in bone resorption mentioned earlier. Early reviews on energy deficiency in athletes suggested the loss of lean mass during periods of energy deficiency may impair performance (Fogelholm, 1994). In addition to compromising performance, the loss of lean mass has been demonstrated to contribute to post-energy deficit hyperphagia, which can lead to a greater regain of fat mass known as the *fat overshoot phenomenon* (Dulloo, Jacquet, Montani, & Schutz, 2015) depicted in Figure 4. This relationship may be particularly detrimental to lean athletes, as lean individuals tend to lose a greater proportion of weight as lean mass (Forbes, 2000). Thus, the loss of lean mass in lean individuals, particularly athletes, in response to energy deficiency may also lead to detrimental outcomes.

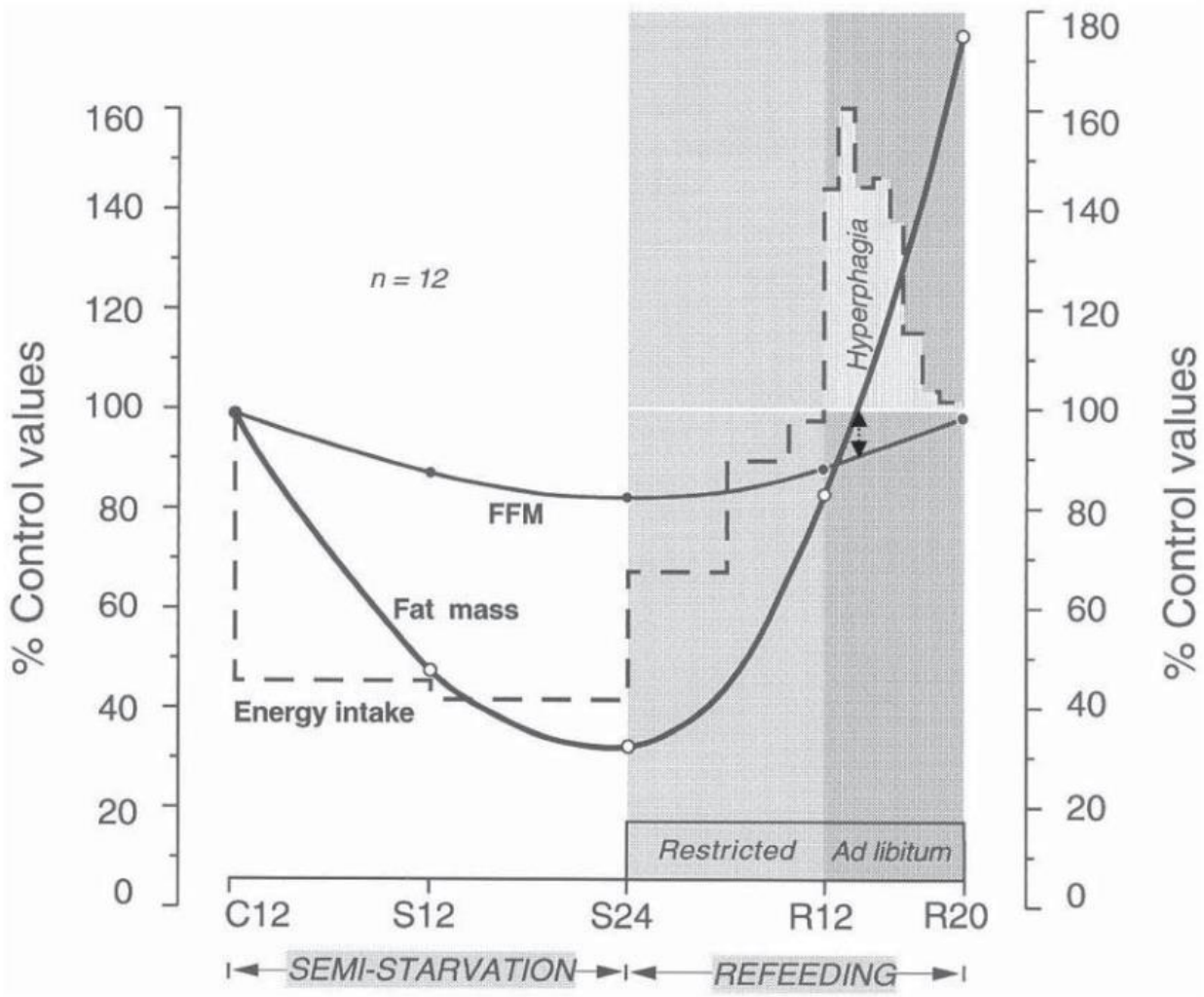

Figure 4. Illustration from Dulloo, Jacquet, Montani & Schutz (2015). Depiction of the fat overshoot phenomenon whereby participants overeat to restore fat-free mass while fat mass continues to increase above pre-weight loss levels. C12, End of 12-week control period; S12, 12 weeks into semi-starvation; S24, 24 weeks into semi-starvation; R12, after 12 weeks of restricted refeeding; R20 after 8 weeks of ad libitum refeeding.

1.3 Rescue of Effects of Energy Deficiency

Now the question is how to prevent, or at least minimize, these negative effects of energy deficiency described above. With some athletes, unintentionally low energy intake during high volume training may create periods of energy deficiency. Under this circumstance, the solution may be increasing nutritional awareness through education of and monitoring by the athlete and staff, as suggested in the IOC Consensus Statement on RED-S (Mountjoy et al., 2018).

However, this dissertation focuses on when periods of energy deficiency cannot be avoided, such as during intentional weight loss and in weight cycling or aesthetic sports and the strategies which can be employed to attenuate these effects.

Regardless of the perspective used to calculate energy status, a decrease in energy intake, an increase in exercise energy expenditure or a combination thereof can create an energy deficiency. Individuals with an energy deficiency experience weight loss, but the compartment of weight lost varies by the method of energy status reduction. Reducing energy intake to induce weight loss canonically results in ~25% of weight lost as lean mass (Weinheimer, Sands, & Campbell, 2010), though some analyses suggest this value may be as high as 40% (Dixon et al., 2015) and, as mentioned earlier, losses of lean mass are generally accepted to be greater in lean populations (Forbes, 2000). By combining a reduction in energy intake with an increase in exercise energy expenditure, the loss of lean mass is halved and inducing weight loss through exercise alone minimizes the loss of lean mass (Weinheimer et al., 2010). These effects of the aforementioned different weight loss modalities are simplistically summarized in Figure 5 (Murphy & Koehler, 2017). In addition to preserving lean mass, the addition of an exercise intervention to a reduction in energy intake similarly preserves bone mineral density during weight loss trials (Yarizadeh et al., 2021).

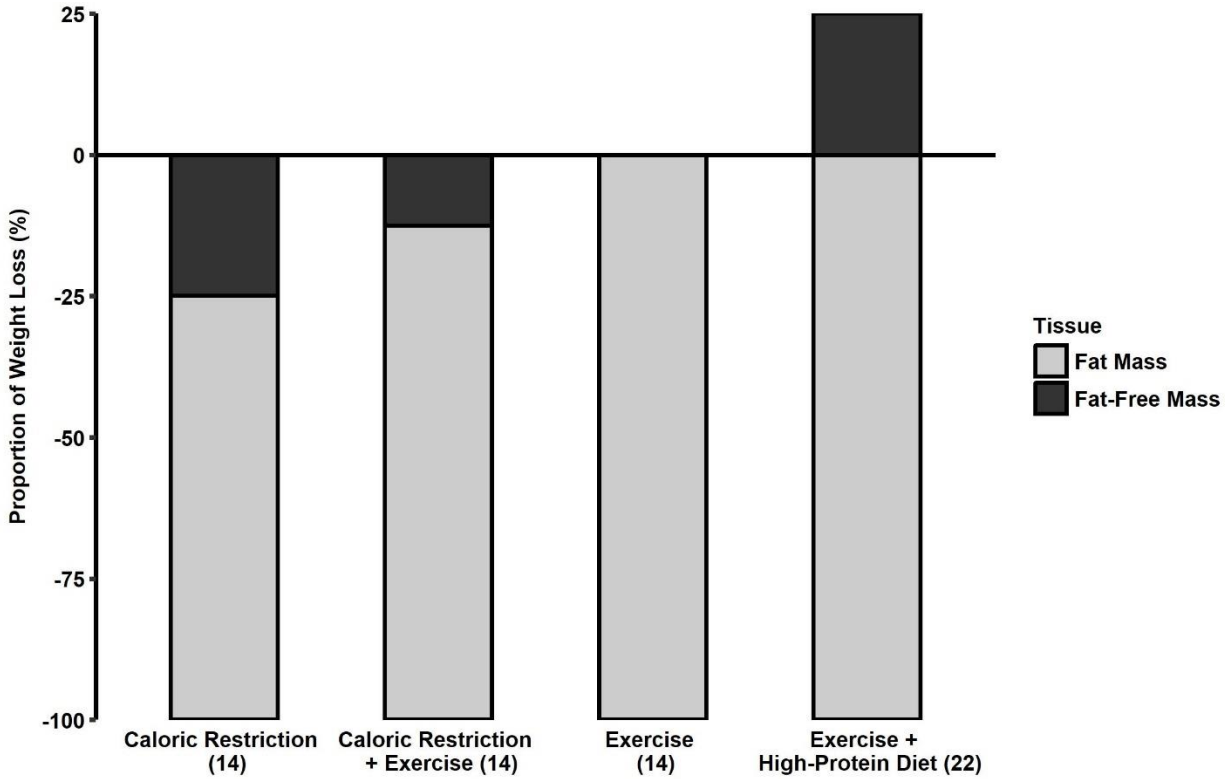

Figure 5. Illustration from Murphy C and Koehler K (2017). Summary of the effects of weight loss modalities on composition of weight lost. References (14) refers to Weinheimer, Sands, & Campbell, 2010 and (22) refers to Longland, Oikawa, Mitchell, Devries, & Phillips, 2016.

However, exercise modalities do not appear to be created equal with regards to these effects on bone mineral density and lean mass. Aerobic exercise is more commonly associated with energy deficiency and weight loss due to its larger energy expenditure compared to resistance exercise. Furthermore, non-weight-bearing aerobic exercise modalities may place individuals at additional risk for adverse bone health outcomes as non-weight-bearing exercise modalities show fewer benefits to skeletal health than their weight-bearing counterparts (Simas, Hing, Pope, & Climstein, 2017). Logically, individuals with the largest energy expenditures who almost exclusively participate in non-weight-bearing activities would then be at the highest risk for adverse bone health outcomes. Among these individuals are athletes such as rowers and cyclists who, specifically, have been shown to display a higher risk for having a low energy availability (Lane, Hackney, et al., 2019) and a high prevalence of low bone mineral density (Viner, Harris, Berning, & Meyer, 2015), likely due to the combination of the aforementioned risk factors. Thus, exercise may be a promising option for mitigating some negative effects of energy deficiency, but specific modalities may be better suited to maximizing these benefits.

While aerobic exercise does preserve lean mass during weight loss compared to non-exercise weight loss interventions (Weiss, Jordan, Frese, Albert, & Villareal, 2017), multiple interventions have established that resistance exercise provides superior protection against the loss of both lean mass and bone mineral density in an energy deficit (Beavers et al., 2017; Villareal et al., 2017). This may be a result of one or both of the greater acute muscle protein synthesis or hormonal responses to higher intensity exercise bouts (Bell, Seguin, Parise, Baker, & Phillips, 2015; Wahl et al., 2013). There are several aspects of the hormonal response to exercise, one of which is the anabolic response of the GH:IGF-1 axis. As mentioned earlier, the integrity of this axis is compromised at rest by the presence of an energy deficient state (Loucks & Thuma, 2003) as are rates of muscle protein synthesis (Areta et al., 2014). However, unlike muscle protein synthesis, which has been shown to be rescued by resistance exercise in an energy deficit (Areta et al., 2014), whether resistance exercise can rescue the sensitivity of the GH:IGF-1 axis has yet to be explored.

Performing resistance exercise is not the only intervention that may assist in attenuating the deleterious effects of energy deficiency. Recent reviews have suggested protein requirements are elevated during periods of energy deficiency, particularly for athletes (Hector & Phillips, 2018). This recommendation results from observations that consumption of a high-protein diet throughout periods of energy deficiency preserves lean mass (Stonehouse et al., 2016) and has been shown to maintain bone mineral density as well (Weaver et al., 2019). These effects may be mediated by a maintenance of IGF-1 sensitivity during energy deficits, as low-protein diets have been shown to impair IGF-1 independent of energy restriction (Smith, Underwood, & Clemmons, 1995). In addition to the independent effects of a high-protein diet in an energy deficit, dietary protein has a long-standing synergistic relationship with resistance training. Post-exercise protein supplementation has been shown to enhance the muscle protein synthesis response to resistance training (Areta et al., 2014) and consumption of a high-protein diet increased lean mass gains in response to four weeks of resistance training in an energy deficit (Longland, Oikawa, Mitchell, Devries, & Phillips, 2016). Thus, additional dietary protein has been shown to be beneficial both alone and in combination with resistance training.

In summary, energy deficiency defined through either a negative energy balance or low energy availability produces a number of negative physiological consequences. Most notably among these are the losses of bone mineral density and skeletal muscle downstream of the hormonal

changes incited by energy deficiency. In order to combat these negative effects, increasing dietary protein consumption and engaging in regular resistance training appear to be promising intervention options.

1.4 Aims

In the following section, the aims of each paper included in this dissertation are presented.

“Low energy availability with and without a high-protein diet suppresses bone formation and increases bone resorption in men: a randomized controlled pilot study” (2.1)
The aim of the first study was to examine whether a high-protein diet could attenuate suppression of metabolic hormones and negative alterations to bone metabolism induced by short-term low energy availability.

“Caloric restriction induces anabolic resistance to resistance exercise” (2.2)
The aim of the second study was to assess whether the dysregulation of the GH:IGF-1 axis observed at rest in response to low energy availability persisted in the face of a bout of resistance exercise and whether the response could be improved by post-exercise protein supplementation.

“Energy Deficiency Impairs Resistance Training Gains in Lean Mass but not Strength: A Meta-Analysis and Meta-Regression” (2.3)
The aim of the third study was to build on our finding from the previous publication (2.2) that the hormonal response to resistance exercise was impaired by low energy availability. This study is the first to systematically assess the impact of energy deficiency on resistance training outcomes.

1.5 Methods

The following section presents the methods used in each of the included publications with an indication of which studies employed the described methods.

1.5.1 Methods for Participant Selection (2.1, 2.2)

Participants for the two clinical trials reported in studies 2.1 and 2.2 were selected based on a number of predefined criteria. In both trials, participants were young, lean and trained.

Individuals between 19 and 30 years old were recruited to participate in study 2.1 to ensure they could recover more effectively between the daily exercise bouts (Woo, Derleth, Stratton, & Levy, 2006) and in study 2.2 because young participants have a larger anabolic response to resistance exercise (Häkkinen, Pakarinen, Newton, & Kraemer, 1998).

Lean men with <20% body fat were recruited to maximize the loss of lean mass in study 2.1 (Forbes, 2000). In study 2.2, lean women with <30% body fat were also recruited to maximize the acute anabolic hormone response to resistance exercise (Thomas et al., 2011). These differing percentage body fat cutoffs were chosen to select participants in the same percentile for their respective sex and ages (Borrud et al., 2010). In study 2.2, recruiting participants with similar body compositions additionally kept the levels of relative strength comparable between participants. Study 2.1 exclusively studied men due to a lack of low energy availability and bone health literature in men compared to women; however, study 2.2 included women as well due to this being the first study to examine the anabolic hormonal response to resistance exercise in an energy deficit.

Trained participants were recruited for study 2.1 to minimize training effects between and within conditions and to aid in ensuring participants could complete and would sufficiently recover between the long, daily training sessions. In study 2.2, trained participants further maximized the anabolic response to exercise (Rubin et al., 2005) and additionally increased the likelihood of consistent performances between the exercise bouts in each condition and ensure participants were comfortable performing fasted bouts of heavy resistance exercise in an energy deficit.

1.5.2 Methods for Quantification of Energy Status (2.1-2.3)

The following subsection presents the methods by which energy status was quantified in the included studies.

1.5.2.1 Prescription of Energy Availability (2.1, 2.2)

In the two clinical trials reported in studies 2.1 and 2.2, participants' energy intake and exercise energy expenditure were all tightly controlled. Participants consumed only the provided liquid diet and exercised only in supervised sessions in the lab. This control permitted the calculation of energy availability in these studies. For the control and intervention energy availabilities, 40 and 15 kcal · kg fat-free mass^{-1} · day^{-1}, respectively, were prescribed. These values were based on previous research showing these levels of energy availability were sufficient to maintain weight and induce weight loss, respectively, as well as produce differences in metabolic hormones such as leptin in a similarly-designed short-term intervention (Koehler et al., 2016).

In the first study (2.1), participants cycled on an ergometer to expend 15 kcal · kg fat-free mass^{-1} · day^{-1}. To achieve our prescribed energy availabilities of 40 and 15 kcal · kg fat-free mass^{-1} · day^{-1}, respectively, after subtracting out the exercise energy expenditure, participants consumed 55 and 30 kcal · kg fat-free mass^{-1} · day^{-1}, respectively. In the second study (2.2), participants did not engage in daily exercise, so we provided them with an energy intake of 40 and 15 kcal · kg fat-free mass^{-1} · day^{-1}, respectively, to achieve the same energy availability.

1.5.2.2 Determination of Energy Deficiency (2.3)

Energy intake and exercise energy expenditure were not tightly controlled in all studies included in the meta-analysis (2.3). Method of energy intake prescription also varied between studies; some studies provided recommendations to reduce energy intake by a certain amount and other studies prescribed diets with a specific energy intake. Furthermore, few studies tightly monitored, and even fewer studies reported any measurement of, energy intake. Additionally, the variation in populations and resistance exercise prescriptions made it difficult to estimate exercise energy expenditure. As such, in order to objectively determine the energy deficit retrospectively, the meta-analysis used changes in energy stored in fat mass with an energy density of 9400 kcal · kg^{-1} (Hall, 2008). This results-oriented approach effectively quantified the

energy deficit achieved by the interventions, which may be preferable to the prescribed energy deficit in interventions with less control over participant behavior.

1.5.3 Methods for Dietary Intervention Prescription (2.1, 2.2)

In studies 2.1 and 2.2, each participant completed three conditions: two at an energy availability of 15 kcal · kg fat-free mass^{-1} · day^{-1} and one control condition at an energy availability of 40 kcal · kg fat-free mass^{-1} · day^{-1}. Due to the discrepancies between exercise energy expenditure detailed both above and below, participants in study 2.1 consumed 30 kcal · kg fat-free mass^{-1} · day^{-1} in the energy deficient conditions and 55 kcal · kg fat-free mass^{-1} · day^{-1} in the energy replete conditions while participants in study 2.2 only consumed 15 and 40 kcal · kg fat-free mass^{-1} · day^{-1} in the energy deficient and energy replete conditions, respectively, to achieve these target energy availabilities.

The diets provided to participants in studies 2.1 and 2.2 were composed of a combination of liquid clinical products (Ensure Plus (4.57 g protein · 100 kcal^{-1}) and Ensure High Protein (10 g protein · 100 kcal^{-1}), Abbott Nutrition) and maltodextrin (Tate & Lyle). Amounts and proportions of the products used were determined by caloric and protein requirements for the condition diets. In study 2.1, the two energy deficient conditions were defined as high-protein and low-protein conditions. The high-protein diet consisted of 1.7 g · kg body weight^{-1} · day^{-1}, the upper limit of the American College of Sports Medicine recommendations for athletes (Rodriguez et al., 2009) which has been shown to be a breaking point for effects of high-protein diets on lean mass (Morton et al., 2018). Meanwhile, the low-protein diet consisted of 0.8 g · kg body weight^{-1} · day^{-1}, the recommended daily allowance for adult protein intake (Food and Nutrition Board, 2005). In study 2.2, we evaluated the effect of post-exercise protein vs carbohydrate supplementation in an energy deficit. To align with previous studies on post-exercise protein supplementation in an energy deficit (Areta et al., 2014) and ensure we provided a sufficient protein bolus to produce a maximal post-exercise anabolic effect (Areta et al., 2013), we provided participants 30 g protein or carbohydrate dissolved in 400 mL water. As such, we moderated the protein intake to 1.2 g · kg body weight^{-1} · day^{-1} to maintain a high-protein diet capable of maintaining lean mass in an energy deficit (Longland et al., 2016), but allowing room for the 30 g post-exercise protein to not exceed the effectiveness breaking point of 1.7 g · kg body weight^{-1} · day^{-1} mentioned earlier (Morton et al., 2018) to increase the likelihood of it conferring some benefit.

As a result of consuming a liquid diet consisting almost entirely of clinical products, consumption of dietary calcium and vitamin D would have differed substantially between participants' habitual diets and the prescribed condition diets. To mitigate these differences, participants consumed calcium and vitamin D supplements throughout the entire study, including washout periods. Calcium and vitamin D provided during each condition were supplemented to make up the difference from the largest amount provided during the study. Supplementation of calcium during washout periods was calculated as the difference between the amount provided within conditions and habitual calcium intake determined using the Brief Calcium Assessment Tool (Yang, Martin, & Boushey, 2010). Vitamin D was supplemented at the maximal amount provided by any condition. Participants were provided all supplements in pill boxes spacing them into 1–3 doses per day depending on the amount supplemented.

1.5.4 Methods for Exercise Intervention Prescription (2.1, 2.2)

In study 2.1, aerobic exercise sessions were performed daily and calibrated to expend 15 kcal · kg fat-free mass^{-1} · day^{-1}. Exercise sessions were calibrated via a preliminary graded exercise test to volitional exhaustion on a cycle ergometer. Participants began the test by cycling at 60 W for 3 minutes and 35 W were added every three minutes until participants could no longer complete a 3-minute stage. Afterwards, the respiratory data were analyzed to identify the intensity at which 60% VO_{2peak} occurred and the energy expenditure per unit time associated with that intensity. Participants cycled each day at the intensity corresponding to 60% VO_{2peak} until an energy expenditure of 15 kcal · kg fat-free mass^{-1} · day^{-1} was achieved.

In study 2.2, participants completed a preliminary resistance exercise bout to familiarize themselves with the barbell rack, available equipment for a self-selected warmup and rhythm of the exercise bouts ahead of the conditions. In the preliminary exercise bout, participants completed sets of 5 repetitions of the barbell back squat exercise following a self-selected warmup, adding weight to each successive set until they indicated ≤ 1 repetitions in reserve following a set or failed to complete a set. Initial loads for the sessions within each condition were chosen as the final set the participant was able to complete. Within the conditions, each session consisted of a self-selected warm-up, 2-5 warm-up sets of the barbell back squat exercise and 5 working sets of the barbell back squat exercise. Loads were adjusted between sets according to the repetitions in reserve reported by participants. When 0 repetitions in

reserve were reported or participants failed to complete a set, the load was decreased. When 1-2 repetitions in reserve were reported, the load was maintained. Finally, if participants reported 3 or more repetitions in reserve, the load was increased. Participants were required to rest for 2 minutes between sets and could rest for up to 5 minutes.

2 Publications

In the following section, the results underlying this thesis are presented. Thematically, each paper aligns under the common banner of the interaction between exercise and energy deficiency. The first two papers additionally consider the interaction of dietary protein with the aforementioned factors. The first study assesses the ability of daily aerobic exercise and dietary protein to preserve lean mass and support bone health (2.1). In the second study, the acute response to a bout of resistance exercise is examined with and without post-exercise protein supplementation (2.2). Finally, in the third paper, a meta-analysis is performed to expand the findings of the second study to long-term outcomes (2.3).

Study 2.1 Title: Low energy availability with and without a high-protein diet suppresses bone formation and increases bone resorption in men: a randomized controlled pilot study

Authors: **Murphy C**, Bilek LD and Koehler K

Abstract: Suppression of IGF-1 and leptin secondary to low energy availability (LEA) may contribute to adverse effects on bone health. Whether a high-protein diet attenuates these effects has not been tested. Seven men completed three five-day conditions operationally defined as LEA (15 kcal · kg fat-free mass (FFM)-1 · day-1) with low protein (LEA-LP; 0.8 g protein · kg body weight (BW)-1), LEA with high protein (LEA-HP; 1.7 g protein · kg BW-1) and control (CON; 40 kcal · kg FFM-1 · day-1, 1.7 g protein · kg BW-1). In all conditions, participants expended 15 kcal · kg FFM-1 · day-1 during supervised cycling sessions. Serum samples were analyzed for markers of bone turnover, IGF-1 and leptin. The decrease in leptin during LEA-LP (-65.6 ± 4.3 %) and LEA-HP (-54.3 ± 16.7 %) was greater than during CON (-25.4 ± 11.4 %; p = .02). Decreases in P1NP (p = .04) and increases in CTX-I (p = .04) were greater in LEA than in CON suggesting LEA shifted bone turnover in favour of bone re-sorption. No differences were found between LEA-LP and LEA-HP. Thus, five days of LEA disrupted bone turnover, but these changes were not attenuated by a high-protein diet.

Contribution: Along with the assistance of my lab mates, I oversaw and coordinated participant scheduling for this clinical trial as well as performed the participant testing and supervised daily exercise sessions. I performed all data analyses, drafted and revised the manuscript and created all figures for the included publication.

2.1 Energy deficiency alters bone turnover with and without a high-protein diet (Study One)

nutrients

MDPI

Article

Low Energy Availability with and without a High-Protein Diet Suppresses Bone Formation and Increases Bone Resorption in Men: A Randomized Controlled Pilot Study

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Abstract: Suppression of insulin-like growth factor 1 (IGF-1) and leptin secondary to low energy availability (LEA) may contribute to adverse effects on bone health. Whether a high-protein diet attenuates these effects has not been tested. Seven men completed three five-day conditions operationally defined as LEA (15 kcal kg fat-free mass (FFM)$^{-1}$·day^{-1}) with low protein (LEA-LP; 0.8 g protein·kg body weight (BW)$^{-1}$), LEA with high protein (LEA-HP; 1.7 g protein·kg BW^{-1}) and control (CON; 40 kcal·kg FFM^{-1}·day^{-1}, 1.7 g protein·kg BW^{-1}). In all conditions, participants expended 15 kcal·kg FFM^{-1}·day^{-1} during supervised cycling sessions. Serum samples were analyzed for markers of bone turnover, IGF-1 and leptin. The decrease in leptin during LEA-LP (−65.6 ± 4.3%) and LEA-HP (−54.3 ± 16.7%) was greater than during CON (−25.4 ± 11.4%; $p = 0.02$). Decreases in P1NP ($p = 0.04$) and increases in CTX-I ($p = 0.04$) were greater in LEA than in CON, suggesting that LEA shifted bone turnover in favour of bone resorption. No differences were found between LEA-LP and LEA-HP. Thus, five days of LEA disrupted bone turnover, but these changes were not attenuated by a high-protein diet.

Keywords: caloric restriction; aerobic exercise; energy deficit

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1. Introduction

Energy availability represents the dietary energy remaining for physiological functions following the deduction of exercise expenditure [1]. At a threshold of 30 kcal·kg fat-free mass (FFM)$^{-1}$·day^{-1} [2], an abundance of hormonal disturbances characterizing low energy availability occurs [1]. In addition to the well-documented suppression of sex hormones [3], key metabolic hormones involved in the regulation of bone metabolism, such as insulin-like growth factor 1 (IGF-1) and leptin, are suppressed by low energy availability [2]. Reductions in IGF-1 secondary to low energy availability have been associated with bone loss [4] and reduced bone mineral density [5] while low leptin levels have been linked to a higher risk of fracture [6].

By definition, energy availability can be lowered through a reduction in energy intake, an increase in exercise energy expenditure or a combination of both. This places individuals with large exercise energy expenditures, such as endurance athletes, at a greater risk of experiencing low energy availability compared to those primarily engaging in training modalities with a lower exercise energy expenditure, such as resistance training [7]. Furthermore, individuals practicing non-weight-bearing exercise modalities have additional risk for adverse bone health outcomes as non-weight-bearing exercise modalities show less benefit to skeletal health than their weight-bearing counterparts [8]. At the intersection of a high exercise energy expenditure and practice of non-weight-bearing exercise are athletes

such as cyclists who, indeed, display an increased risk for low energy availability [9] and a high prevalence of low bone mineral density [10] likely originating from this combination of risk factors. Thus, for these and other athletes at risk for experiencing periods of low energy availability, additional measures to mitigate the harmful effects of low energy availability on bone health are needed.

One strategy with underexplored potential is that of dietary protein. During periods of low energy availability, protein requirements, particularly for athletes, are elevated [11]. Increased dietary protein preserves lean mass, which is positively associated with bone mineral density [12]. Indeed, a high-protein diet has been shown to preserve lean mass and bone mineral density similar to an energy balance control during a six-month weight loss intervention [13]. Maintenance of lean mass and bone mineral density during periods of low energy availability in athletes may improve performance capacity and reduced risk for future injury, or even osteoporosis later in life [14,15]. Mechanistically, high protein intakes may exert these protective effects by attenuating reductions in IGF-1 characteristic of low energy availability exposure. This hypothesis comes from the observation that low protein intakes have been shown to suppress IGF-1 even without the presence of energy restriction [16].

Therefore, the purpose of our pilot study was to first confirm the effects of low energy availability induced by a combination of dietary energy restriction and exercise energy expenditure on hormones such as IGF-1 and leptin and downstream markers of bone turnover using a non-loading form of aerobic exercise, namely cycling. Previous research examining the impact of low energy availability induced via exercise energy expenditure on bone markers has been limited to the use of weight-bearing running exercise [17,18]. Additionally, we wanted to explore whether increased dietary protein preserves upstream signals from IGF-1 or leptin and blunts the bone turnover marker response during short-term low energy availability. While IGF-1 has been shown to respond to protein restriction independent of energy restriction [16], leptin is tightly linked to energy availability and likely will not respond to increased protein intake without a change in energy availability. Thus, we hypothesize that low energy availability will decrease circulating IGF-1 and leptin as well as increase bone resorption and decrease bone formation. We anticipate that a high-protein diet will attenuate the effects of low energy availability on IGF-1 and bone turnover markers, but not leptin.

2. Materials and Methods

2.1. Study Design

The present randomized, single-blind repeated measures crossover pilot study consisted of three five-day conditions (Table 1), a sufficient duration for detecting changes in metabolic hormones and markers of bone turnover in response to low energy availability [18]. Two conditions restricted energy intake to 30 kcal·kg fat-free mass (FFM)$^{-1}$·day^{-1} (LEA). In one LEA condition (LEA low protein; LEA-LP), participants consumed 0.8 g·kg body weight (BW)$^{-1}$·day^{-1} protein in accordance with the recommended daily allowance. The other LEA condition (LEA high protein; LEA-HP) provided participants 1.7 g·kg BW^{-1}·day^{-1} protein, an amount reflecting the upper limit of protein recommended for athletes by the American College of Sports Medicine [19] which has been shown to preserve lean mass during energy restriction [20]. Participants also underwent a condition which provided them 55 kcal·kg FFM^{-1}·day^{-1} and 1.7 g·kg BW^{-1}·day^{-1} protein, operationally defined as the control condition (CON). Participants expended 15 kcal·kg FFM^{-1}·day^{-1} in supervised exercise sessions during all conditions. This resulted in a net energy availability after daily exercise of 15 kcal·kg FFM^{-1}·day^{-1} in the LEA conditions and 40 kcal·kg FFM^{-1}·day^{-1} in the CON condition. These levels of energy availability have induced significant weight loss and maintained weight, respectively, in a similar intervention [21]. Participants were randomly assigned to one of six condition sequences by a random number generator and completed a washout period of at least two weeks between conditions during which they continued habitual exercise and dietary practices. This duration of

washout period is slightly longer than what has previously been used (10 days) to recover body weight and metabolic hormones following short-term exposure to an LEA of 15 kcal·kg FFM^{-1}·day^{-1} [21]. The study was approved by the University of Nebraska—Lincoln's Institutional Review Board (IRB#15895; Approved 17 March 2016) and registered at www.clinicaltrials.gov (NCT02945410; accessed on 19 December 2019).

Table 1. Energy and protein characteristics of the three conditions.

Condition	LEA-LP	LEA-HP	CON
Energy Intake [1]	30	30	55
Exercise Energy Expenditure [1]	15	15	15
Energy Availability [1]	15	15	40
Protein Intake [2]	0.8	1.7	1.7

Abbreviations: LEA-LP, low energy availability with low protein; LEA-HP, low energy availability with high protein, CON, control. [1] units: kcal·kg FFM^{-1}·day^{-1}; [2] units: g·kg body weight^{-1}.

2.2. *Participants*

We conducted the pilot study between 1 September 2016 and 15 January 2018. Participants were recruited from campus and other local recreation sites via flyers, emails to campus sports clubs and social media posts. Participants were nonsmokers between 19 and 30 years old with a normal body fat percentage (<20%) as measured by skinfold measurement (CE 0120, Harpenden, UK) and completed ≥4 h of purposeful aerobic exercise per week for six months prior to beginning the study. We selected young participants for the study to ensure participants could recover quickly from the high physical demands of the study while lean participants lose greater amounts of lean mass during weight loss [22], which maximized effect sizes. Recruiting trained participants reduced training effects of the interventions and ensured participants would be able to complete and recover from daily exercise sessions. Compliance to these inclusion criteria was confirmed during an initial screening visit to the laboratory after the informed consent was signed.

2.3. *Preliminary Testing*

During preliminary testing, participants had their height and weight taken by an electronic stadiometer (222 and 769, SECA, Hamburg, Germany) and their body composition assessed by bioimpedance analysis (Quadscan 4000, BodyStat, UK). Participants also completed a graded exercise test on a cycle ergometer (LC6, Monark HB, Sweden) to assess peak oxygen consumption (VO2peak). Participants began cycling at 60 W for 3 min and the intensity was increased by 35 W every 3 min until volitional exhaustion, which required at least 3 of the following: (1) cadence < 60 rpm, (2) respiratory exchange ratio ≥ 1.1, (3) heart rate ≥ 90% of age-predicted maximum (220-age), (4) plateau in oxygen uptake despite increasing workload, (5) rating of perceived exertion ≥ 19. Respiratory data were analyzed by a metabolic cart (QUARK CPET, COSMED, USA) and used to determine the intensity corresponding to 60% VO2peak.

2.4. *Diet Preparation*

Participants were provided all food consumed during each five-day condition. Diets consisted of an individually tailored combination of clinical products (Ensure Plus; 4.57 g protein·100 kcal^{-1} and Ensure High Protein; 10 g protein·100 kcal^{-1}, both Abbott Nutrition, USA) and maltodextrin (Tate and Lyle, UK). The LEA-LP diet consisted of Ensure Plus providing 0.8 g·kg BW^{-1} protein and maltodextrin added to achieve a caloric intake of 30 kcal·kg FFM^{-1}·day^{-1}. In the remaining conditions, maltodextrin consumption was matched with LEA-LP and the required amounts of the two clinical products were calculated to obtain 1.7 g·kg BW^{-1}·day^{-1} and either 30 kcal·kg FFM^{-1}·day^{-1} (LEA-HP) or 55 kcal·kg FFM^{-1}·day^{-1} (CON). Participants consumed their maltodextrin during daily exercise bouts dissolved in 800 mL water·hour^{-1} exercise with 1.2 g sodium chloride·L^{-1} to attenuate dehydration and enhance palatability [23]. We supplied a wholly liquid diet

from products used in previous interventions [2] to accurately measure intake and blind participants by matching dietary volume between conditions via dilutions with water. Participants were required to consume their food in ≥3 meals spread throughout the day and each participant consumed the same number of meals every day throughout the entire study. During the conditions, participants were permitted to consume non-caloric beverages, but were asked to record consumption of these products.

2.5. Supplementation

To mitigate differences in calcium and vitamin D consumption, we supplemented participant intake of these micronutrients throughout the entire study, including washout periods. Calcium and vitamin D provided during each condition were supplemented to make up the difference from the largest amount provided during the study. Supplementation of calcium during washout periods was calculated as the difference between the amount provided within conditions and habitual calcium intake determined using the Brief Calcium Assessment Tool [24]. Vitamin D was supplemented at the maximal amount provided by any condition. Participants were provided all supplements in pill boxes spacing them into 1–3 doses per day depending on number of supplements consumed.

2.6. Daily Exercise Prescription

Daily aerobic exercise sessions on the cycle ergometer were calibrated to expend 15 kcal·kg FFM^{-1}·day^{-1} at the power output corresponding to 60% of VO2peak achieved during the preliminary graded exercise test. Duration of the daily exercise sessions was calculated by dividing the target energy expenditure of the exercise session by the rate of energy expenditure at the determined power output. Additional exercise and intense physical activity were prohibited. Compliance was measured via a waist-worn accelerometer (ActiLife G3TX+, ActiGraph, USA).

2.7. Measurements and Assessments

All measurements were performed in an identical order before (pre) and after (post) each five-day condition. Participants reported to the laboratory between 0700 and 0800 following an overnight fast of at least 12 h. Body weight and composition were measured as reported for preliminary assessments. Then a blood sample was collected from the antecubital vein. Serum aliquots were stored at −80 °C until analysis.

Commercially available assays were used to measure serum concentrations of IGF-1 [R&D Systems, Minneapolis, MN, USA], insulin-like growth factor binding protein-3 (IGFBP-3) [R&D Systems, USA], Leptin [Mediagnost, Reutlingen, Germany], CTX-I [AB-Clonal, Woburn, MA, USA], P1NP [Cloud Clone, Katy, TX, USA] and sclerostin [Biomedica, Mountain View, CA, USA]. In-house intraassay variabilities for each assay were 3.24% (IGF-1, sensitivity: 0.056 ng/mL), 3.37% (IGFBP-3, sensitivity: 0.14 ng/mL), 1.92% (Leptin, sensitivity: 0.25 µg/L), 7.66% (CTX-I, sensitivity: 0.1 ng/mL), 6.69% (P1NP, sensitivity: 17.71 pg/mL) and 7.30% (sclerostin, sensitivity: 3.17 pmol/L). The IGF-1: IGFBP-3 Ratio (IGFR) was calculated by multiplying the ng/mL concentrations of IGF-1 and IGFBP-3 provided from the assay by 0.13 and 0.036, respectively, to obtain molar concentrations and dividing the molar concentration of IGF-1 by the molar concentration of IGFBP-3 [25].

2.8. Statistical Analyses

Changes from pre- to post-condition were expressed in the original units for body composition outcomes and IGFR and percentage changes for markers of bone turnover (P1NP, CTX-I and sclerostin), IGF-1 and leptin. Prior to analysis, all data were examined for outliers, defined as values greater than three standard deviations away from the mean, and assessed for normality using the Shapiro–Wilk test. Following the removal of one outlier in the IGF-1 data, all data were determined to be normally distributed. All outcomes were first analyzed for the effect of LEA by ANOVA. Post hoc, one-sided paired *t*-tests were then performed on hypothesized differences between LEA-LP and LEA-HP. Sample size

was determined based on literature reporting changes in IGF-1 following the reduction in energy availability to 10 kcal·kg FFM^{-1}·day^{-1} for five days [2]. From this data, we anticipated an effect size of 1.1 and a sample size of $n = 7$ was deemed sufficient to detect differences with a power of 0.80. All statistical analysis was performed using R (R Core Team, Version 3.6). Unless otherwise stated, all data in text and figures are reported as mean ± standard error of the mean (SEM). We defined statistical significance as $p < 0.05$.

3. Results

3.1. Participant Characteristics and Compliance

Of the 15 participants allocated to an intervention, 10 participants completed at least one condition and seven participants finished all three conditions (Supplementary Figure S1). At baseline, the seven completers were 23.9 ± 1.5 years of age, weighed 86.9 ± 2.9 kg with 13.4 ± 2.0% body fat and had an average VO2peak of 42.6 ± 2.4 mL·kg^{-1}·min^{-1}. Completers did not differ from participants allocated to an intervention but unable to complete all conditions on any of the aforementioned variables (all $t > 1.28$, $p > 0.20$).

During each condition, completers exercised at an intensity of 124 ± 12 Watts for 115 ± 10 minutes to expend 15 kcal·kg FFM^{-1}·day^{-1}. All participants attended 100% of their prescribed exercise sessions in each condition. Participants exchanged their empty beverage containers from the previous day for their next days' meals at each exercise session in addition to completing a dietary intake log of the beverages for each condition. Based on these procedures, dietary compliance was 100%.

3.2. Body Weight and Composition

Completers lost similar amounts of body weight during LEA-HP (−2.27 ± 0.50 kg) and LEA-LP (−2.13 ± 0.30 kg) but not CON (−0.01 ± 0.33 kg; $F = 19.05$, $p = 0.002$). Due to technical difficulties, complete body composition data were only available for five participants (Figure 1). These five completers lost more fat mass (FM) (−1.14 ± 0.23 kg and −0.92 ± 0.18 kg vs. −0.10 ± 0.40 kg; $F = 8.76$, $p = 0.02$) and dry lean mass (DLM) (−0.36 ± 0.07 kg and −0.33 ± 0.07 kg vs. −0.06 ± 0.05 kg; $F = 10.44$, $p = 0.01$) in LEA conditions compared to CON. Losses of FM (mean difference = −0.22, $t = -1.20$, $p = 0.15$) and DLM (mean difference = −0.04, $t = -0.52$, $p = 0.69$) were not significantly different between LEA-HP and LEA-LP.

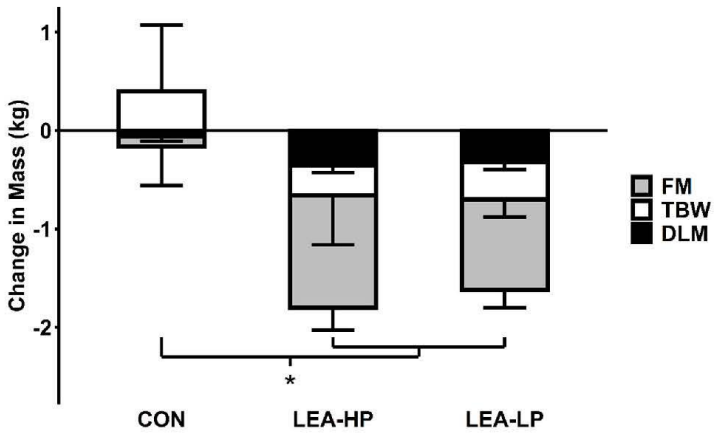

Figure 1. Changes in body composition by condition. Abbreviations: FM, fat mass; TBW, total body water; DLM, dry lean mass; CON, control; LEA-HP, low energy availability with high protein; LEA-LP, low energy availability with low protein. * indicates $p < 0.05$ LEA vs. CON for each compartment.

3.3. Leptin, IGF-1 and IGFR

Pre- and post-condition measurements for all hormonal outcomes are reported in Table 2. Decreases in leptin were greater during LEA-HP (−54.3 ± 16.7%) and LEA-LP (−65.6 ± 4.3%) conditions than during CON (−25.4 ± 11.4%; $F = 7.50$, $p = 0.02$). The differences in changes in IGF-1 between LEA (HP: −8.1 ± 7.3%; LP −11.8 ± 5.4%) and CON (2.9 ± 9.4%; $F = 2.42$, $p = 0.14$) did not achieve statistical significance (Figure 2). Differences in IGFR changes followed the same pattern as IGF-1 ($F = 2.37$, $p = 0.15$). Due to the lack of difference between LEA and CON conditions, the difference between LEA-HP and LEA-LP was not tested.

Table 2. Biomarker responses to each condition.

Hormone	Condition	Pre	Post	% Change	LEA vs. CON *p* Value
P1NP (µg/L)	LEA-LP	90.9 ± 11.7	77.9 ± 12.2	−14.9 ± 6.5%	0.04
	LEA-HP	85.4 ± 11.8	61.7 ± 7.6	−24.8 ± 6.2%	
	CON	85.0 ± 8.2	80.8 ± 8.7	−4.4 ± 5.5%	
CTX-I (ng/mL)	LEA-LP	1.30 ± 0.16	1.39 ± 0.19	6.9 ± 6.2%	0.04
	LEA-HP	1.22 ± 0.10	1.23 ± 0.10	0.2 ± 2.1%	
	CON	1.47 ± 0.19	1.32 ± 0.14	−8.3 ± 3.9%	
Sclerostin (pmol/L)	LEA-LP	31.4 ± 2.9	36.4 ± 4.7	15.0 ± 8.4%	0.81
	LEA-HP	30.4 ± 3.6	30.8 ± 3.5	3.2 ± 6.9%	
	CON	28.6 ± 5.4	30.3 ± 5.6	6.6 ± 9.5%	
Leptin (µg/L)	LEA-LP	3.28 ± 1.77	1.44 ± 0.89	−65.5 ± 4.4%	0.02
	LEA-HP	2.50 ± 1.21	1.23 ± 0.75	−54.3 ± 16.7%	
	CON	3.03 ± 1.24	2.57 ± 1.51	−25.4 ± 11.4%	
IGF-1 (ng/mL)	LEA-LP	228 ± 30	200 ± 27	−11.8 ± 5.4%	0.14
	LEA-HP	202 ± 29	180 ± 21	−8.1 ± 7.3%	
	CON	225 ± 33	221 ± 20	2.9 ± 9.4%	
IGFBP-3 (ng/mL)	LEA-LP	2418 ± 131	2281 ± 111	−5.2 ± 3.6%	0.61
	LEA-HP	2282 ± 186	2311 ± 80	4.1 ± 7.3%	
	CON	2612 ± 132	2502 ± 117	−3.9 ± 2.7%	
IGFR (no units)	LEA-LP	0.34 ± 0.05	0.32 ± 0.04	-	0.15
	LEA-HP	0.34 ± 0.07	0.28 ± 0.04	-	
	CON	0.32 ± 0.06	0.32 ± 0.04	-	

P1NP, CTX-I, Sclerostin, Leptin ($n = 7$); IGF-1, IGFBP-3, IGFR ($n = 6$). Abbreviations: P1NP, procollagen type I N-terminal propeptide; CTX-I, type I collagen cross-linked C-telopeptide; IGF1, insulin-like growth factor-1; IGFBP3, insulin-like growth factor binding protein 3; IGFR, insulin-like growth factor ratio; LEA-LP, low energy availability with low protein; LEA-HP, low energy availability with high protein; CON, control.

3.4. Markers of Bone Turnover

As shown in Figure 3, P1NP decreased to a greater extent during LEA-HP (−24.8 ± 6.2%) and LEA-LP (−14.9 ± 6.5%) conditions than during CON (−4.4 ± 5.5%; $F = 4.95$, $p = 0.04$). CTX-I increased to a greater extent during LEA-HP (0.2 ± 2.1%) and LEA-LP (6.9 ± 6.2%) conditions than during CON (−8.3 ± 3.9%; $F = 5.00$, $p = 0.04$). However, changes in Sclerostin were not different between LEA and CON (LEA-HP, 3.2 ± 6.9%; LEA-LP, 15.0 ± 8.4%; CON, 6.6 ± 9.5%; $F = 0.06$, $p = 0.81$). None of the changes in the turnover markers above achieved statistically significant differences between LEA-HP and LEA-LP (P1NP, $t = -1.01$, $p = 0.82$; CTX-I, $t = -0.91$, $p = 0.16$; sclerostin, $t = -0.94$, $p = 0.19$, respectively).

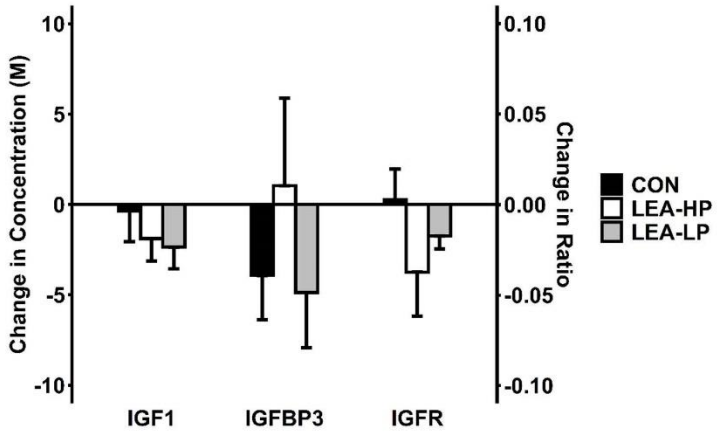

Figure 2. Changes in IGFR and its components by condition. Abbreviations: CON, control; LEA-LP, low energy availability with low protein; LEA-HP, low energy availability with high protein; M, molar concentration; IGF1, insulin-like growth factor-1; IGFBP3, insulin-like growth factor binding protein 3; IGFR, insulin-like growth factor ratio.

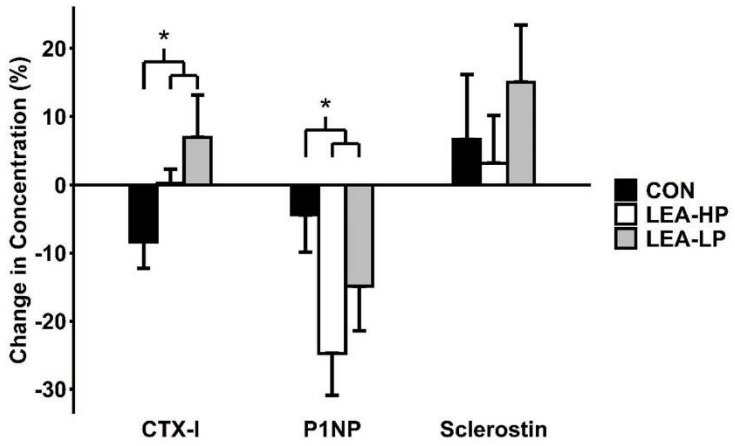

Figure 3. Changes in markers of bone turnover by condition. Abbreviations: CTX-I, type I collagen cross-linked C-telopeptide; P1NP, procollagen type I N-terminal propeptide; CON, control; LEA-LP, low energy availability with low protein; LEA-HP, low energy availability with high protein. * indicates $p < 0.05$ LEA vs. CON.

4. Discussion

The present intervention examined the effects of acute low energy availability exposure on upstream metabolic hormones leptin and IGF-1 as well as downstream markers of bone turnover in men performing daily non-weight-bearing exercise. To our knowledge, the present pilot study is the first controlled low energy availability intervention to explore the ability of a high-protein diet to attenuate the effects of low energy availability on bone turnover. Our results show five days of low energy availability achieved through dietary restriction and daily cycling exercise decreased circulating levels of leptin, but not IGF-1,

and reduced bone formation and increased bone resorption. However, the high-protein diet showed a limited ability to blunt these responses.

In agreement with our hypotheses, leptin declined in response to low energy availability. This supports reductions previously observed in lean men [21], sedentary women [2] and a pooled analysis of active men and women [18] in response to low energy availability exposure. Positive associations between leptin and bone mineral density have been widely reported and a growing body of literature supports both direct and indirect mechanisms of action responsible for this observation [26]. Whether reductions in leptin per se are responsible for the shift in bone turnover to favour bone resorption during low energy availability has not been examined. However, we also speculate that leptin makes a poor target for intervention given how robustly it responds to low energy availability, regardless how energy availability is reduced.

In contrast, we were unable to observe a significant effect of low energy availability on IGF-1 or IGFR. Seminal work on the threshold for disruption of hormones showed that IGF-1 decreased in a dose-dependent fashion across 30, 20 and 10 kcal·kg FFM^{-1}·day^{-1} in sedentary women [2]. This finding has been supported in a pooled group of men and women by the work of Papageorgiou et al. [18]. However, previous work in lean, trained men similar to our own population did not observe significant changes in IGF-1 at an energy availability of 15 kcal·kg FFM^{-1}·day^{-1} [21]. The less consistent findings in men compared to women suggest that IGF-1 may not respond as robustly to low energy availability in men as in women. Potential explanations for this observation include increased peripheral synthesis of IGF-1 by skeletal muscle [27] due to a greater appendicular lean mass in men or a divergent relationship between IGF-1 and estrogen vs. testosterone [28].

Furthermore, a high-protein diet did not beneficially impact the IGF-1 response to low energy availability. We previously showed a single bolus of 30 g whey protein given post-resistance exercise was unable to protect against the decline in IGF-1 observed during low energy availability [29]. We speculated that a more consistent delivery of increased dietary protein (e.g., a high-protein diet) was needed to observe effects of protein on IGF-1. While we were unable to observe such effects in the present study, a one-year weight loss intervention in postmenopausal women did find that a high-protein diet elevated both IGF-1 and IGFBP-3 in addition to improving bone mineral density at several sites [30]. We speculate the relationships between dietary protein, IGF-1 and bone health may be mediated by the preservation of lean mass—which is associated with bone mineral density [12]—and require a sufficient duration for differences in lean mass preservation to appear in order to manifest. However, the aforementioned one-year intervention did not observe any differences in lean mass changes between their protein intakes [30]. Thus, it remains to be determined what role dietary protein plays in moderating changes in IGF-1 and whether this causally influences bone health.

Low energy availability significantly impaired bone formation, indicated by circulating P1NP, and elevated bone resorption, indicated by circulating CTX-I. P1NP has previously been shown to decrease in response to low energy availability induced by daily running exercise [17,18]. This agreement between our results suggests that our choice of a non-weight-bearing exercise modality likely did not contribute to these findings. Previous research has reported a strong correlation (r = 0.97) between P1NP and IGF-1 [17]. In the present study, our pre- and post-condition values were only moderately correlated (r = 0.66). However, it is promising that we observed a similar P1NP response to LEA in the present study (~15–25%) to that reported in previous research by Zanker and Swaine (15%) [17] and Ihle and Loucks (20–25%) [4]. Though we observed statistically significant differences for CTX-I between LEA and CON, the magnitude of these changes is below reported ranges for intraindividual variability (~10%) [30]. However, our strict control over diet, exercise, physical activity as well as calcium and Vitamin D likely reduced the potential intraindividual variability in the present study.

In the present intervention, consumption of a high-protein diet did not appear to protect against reduced P1NP but showed signs of a protective effect on CTX-I we were

not adequately powered to detect. Our observations match that of longer duration interventions which found no effect of a high-protein diet on P1NP [31] and a protective effect on CTX-I [32]. This combination of findings is interesting given that the effects of protein on other tissues, such as lean mass, are often mediated by anabolic and not anti-catabolic effects.

Unlike P1NP and CTX-I, sclerostin did not appear to be impacted by low energy availability in the present intervention. Previously, we reported an increase in sclerostin following just two days of low energy availability and inactivity which was attenuated by performing a bout of resistance exercise on the third day [29]. The absence of a significant increase in the present study suggests that even non-weight-bearing aerobic exercise, when performed daily, may be sufficient to prevent significant elevations in sclerostin during short periods of low energy availability. This is a surprising finding given that sclerostin is produced in response to mechanical unloading and suppressed by mechanical loading [33]. However, sclerostin has previously been shown to respond to both weight-bearing and non-weight-bearing exercise stimuli when both P1NP and CTX-I did not [34]. Thus, it appears that sclerostin may respond more robustly to the exercise stimulus, even during low energy availability, than the standard markers, P1NP and CTX-I, though additional data are needed to support this hypothesis.

Our intervention is one of a limited number of diet and exercise interventions designed to prospectively study the effects of low energy availability exposure. We strictly controlled energy availability through supplying participants with all meals and supervising exercise bouts each day of the intervention. All of this was done to study the effects in men alone. We chose this study population due to the scarcity of research on the effects of low energy availability in men and to help clarify some of the less consistent findings in men compared to women. Albeit small, the sample size (n = 7) is similar to previous controlled LEA experiments (n = 6–11) [2,17,18,21,29] and our use of a crossover design adequately powered the pilot study to detect changes similar to those seen in previous studies as a result of LEA [2]. Additional research, particularly in larger studies, is still needed to investigate the effects of high-protein diets during LEA. Nonetheless, the present study supports existing low energy availability literature by reinforcing the effects of low energy availability on markers of bone turnover and introducing the potential of high-protein diets to augment the effects of exercise in the context of low energy availability.

5. Conclusions

In the present pilot study, low energy availability achieved through a combination of energy restriction and daily cycling exercise reduced circulating levels of leptin, but not IGF-1, in men. Despite this, the combined reduction in bone formation and elevation in bone resorption still signaled a shift in bone turnover favoring resorption. Consuming a high-protein diet during low energy availability did not significantly attenuate these effects. Additional research is needed to further explore the differential responses of IGF-1 to low energy availability between men and women and further investigate the potential of high-protein diets as a strategy to attenuate these deleterious effects.

Supplementary Materials: The following are available online at https://www.mdpi.com/2072-6643/13/3/802/s1, Figure S1: CONSORT 2010 Flow diagram.

Author Contributions: Conceptualization, K.K.; methodology, K.K.; formal analysis, C.M.; investigation, C.M.; resources, L.D.B. and K.K.; data curation, C.M.; writing—original draft preparation, C.M.; writing—review and editing, L.D.B. and K.K.; visualization, C.M.; supervision, K.K.; project administration, K.K.; funding acquisition, K.K. All authors have read and agreed to the published version of the manuscript.

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Study 2.2 Title: Caloric restriction induces anabolic resistance to resistance exercise

Authors: **Murphy C** and Koehler K

Abstract: **Purpose** Weight loss can result in the loss of muscle mass and bone mineral density. Resistance exercise is commonly prescribed to attenuate these effects. However, the anabolic endocrine response to resistance exercise during caloric restriction has not been characterized. **Methods** Participants underwent 3-day conditions of caloric restriction (15 kcal kg FFM^{-1}) with post-exercise carbohydrate (CRC) and with post-exercise protein (CRP), and an energy balance control (40 kcal kg FFM^{-1}) with post-exercise carbohydrate (CON). Serial blood draws were taken following five sets of five repetitions of the barbell back squat exercise on day 3 of each condition. **Results** In CRC and CRP, respectively, growth hormone peaked at 2.6±0.4 and 2.5±0.9 times the peak concentrations observed during CON. Despite this, insulin-like growth factor-1 concentrations declined 18.3±3.4% in CRC and 27.2±3.8% in CRP, which was greater than the 7.6±3.6% decline in CON, over the subsequent 24 h. Sclerostin increased over the first 2 days of each intervention by 19.2±5.6% in CRC, 21.8±6.2% in CRP and 13.4±5.9% in CON, but following the resistance exercise bout, these increases were attenuated and no longer significant. **Conclusion** During caloric restriction, there is considerable endocrine anabolic resistance to a single bout of resistance exercise which persists in the presence of post-exercise whey protein supplementation. Alternative strategies to restore the sensitivity of insulin-like growth factor-1 to growth hormone need to be explored.

Contributions: I was involved with the design and conceptualization of this clinical trial, in addition to performing participant testing, recruitment, scheduling and coordination. For the manuscript, I performed all analyses, drafted the original manuscript, revised the manuscript and designed all figures.

2.2 Energy deficiency alters GH:IGF-1 axis response to resistance exercise (Study Two)

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ORIGINAL ARTICLE

Caloric restriction induces anabolic resistance to resistance exercise

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Abstract

Purpose Weight loss can result in the loss of muscle mass and bone mineral density. Resistance exercise is commonly prescribed to attenuate these effects. However, the anabolic endocrine response to resistance exercise during caloric restriction has not been characterized.

Methods Participants underwent 3-day conditions of caloric restriction (15 kcal kg FFM^{-1}) with post-exercise carbohydrate (CRC) and with post-exercise protein (CRP), and an energy balance control (40 kcal kg FFM^{-1}) with post-exercise carbohydrate (CON). Serial blood draws were taken following five sets of five repetitions of the barbell back squat exercise on day 3 of each condition.

Results In CRC and CRP, respectively, growth hormone peaked at 2.6 ± 0.4 and 2.5 ± 0.9 times the peak concentrations observed during CON. Despite this, insulin-like growth factor-1 concentrations declined 18.3 ± 3.4% in CRC and 27.2 ± 3.8% in CRP, which was greater than the 7.6 ± 3.6% decline in CON, over the subsequent 24 h. Sclerostin increased over the first 2 days of each intervention by 19.2 ± 5.6% in CRC, 21.8 ± 6.2% in CRP and 13.4 ± 5.9% in CON, but following the resistance exercise bout, these increases were attenuated and no longer significant.

Conclusion During caloric restriction, there is considerable endocrine anabolic resistance to a single bout of resistance exercise which persists in the presence of post-exercise whey protein supplementation. Alternative strategies to restore the sensitivity of insulin-like growth factor-1 to growth hormone need to be explored.

Keywords Energy deficit · Energy availability · Weightlifting · Strength training · Growth hormone · Sclerostin

Abbreviations

AUC	Area under the curve
BW	Body weight
CON	Control condition
CR	Caloric restriction conditions
CRC	Caloric restriction with carbohydrate condition
CRP	Caloric restriction with protein condition
GH	Growth hormone
IGF-1	Insulin-like growth factor-1
P1NP	N-terminal propeptide of type-1 collagen
RIR	Repetitions-in-reserve

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Introduction

While weight loss is necessary to combat obesity and its associated comorbidities, it may negatively impact both the muscular (Weinheimer et al. 2010) and skeletal (Ensrud et al. 2018) systems. Weight loss consistently reduces muscle protein synthesis (Hector et al. 2018; Pasiakos et al. 2013) and has been found to increase muscle protein breakdown (Carbone et al. 2014). These changes parallel the suppression of bone formation (Ihle and Loucks 2004) and elevation of bone resorption (Ihle and Loucks 2004) during weight loss. Thus, exercise is often recommended to attenuate the insults of caloric restriction to the musculoskeletal system (Weinheimer et al. 2010). Though both aerobic and resistance exercise have been shown to preserve lean mass (Weiss et al. 2017; Sardeli et al. 2018) and bone mineral

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density (Armamento-Villareal et al. 2012; Villareal et al. 2006) during calorie-restricted weight loss, some evidence suggests that resistance training may be superior for preserving lean mass (Clark 2015; Villareal et al. 2017) and bone mineral density (Beavers et al. 2017; Armamento-Villareal et al. 2019), potentially due to the larger anabolic endocrine response generated by higher intensity exercise protocols (Wahl et al. 2013).

However, the response of anabolic hormones to resistance exercise may be altered under caloric restriction. At energy balance, growth hormone (GH) secretion from the anterior pituitary stimulates insulin-like growth factor-1 (IGF-1) production, primarily in the liver (Vottero et al. 2013). In turn, the resulting increase in IGF-1 provides negative feedback to the hypothalamus and anterior pituitary, reducing the production of GH releasing hormone and GH, respectively (Vottero et al. 2013). Previous research has demonstrated caloric restriction disrupts the GH:IGF-1 axis, such that increasing GH secretion does not stimulate IGF-1 production and, in turn, there is no subsequent negative feedback to reduce GH production (Fazeli and Klibanski 2014). These alterations occur in a dose-dependent fashion, such that higher levels of caloric restriction produce greater increases in GH and reductions in IGF-1 compared to lower levels of caloric restriction and energy balance (Loucks and Thuma 2003). This dysregulated pairing of increased GH and decreased IGF-1 has been termed growth hormone resistance (Fazeli and Klibanski 2014) and represents a specific form of anabolic resistance. However, whether this dysregulation persists in the face of a potent anabolic stimulus, such as resistance training, has not been investigated.

The responses of systemic anabolic factors, such as GH and IGF-1, warrant consideration as both hormones play significant roles in the development of the skeletal system (Tritos and Klibanski 2016). The reduction in IGF-1 during caloric restriction, specifically, has been associated with bone loss (De Souza and Williams 2005), and bone lost during weight loss is not easily restored (Villalon et al. 2011). Previous studies have used short-term caloric restriction to induce substantial changes in markers of bone turnover (Papageorgiou et al. 2017; Loucks and Thuma 2003). Changes in markers of bone turnover appear before noticeable changes in bone mineral density can be observed (Fujimura et al. 1997), but have been shown to parallel changes in bone mineral density in long-term studies (Villareal et al. 2016). Therefore, markers of bone turnover can serve as reliable indicators of the shift in bone metabolism during short-term interventions. Investigating the short-term effects of resistance exercise on markers of bone turnover during caloric restriction is an important first step towards refining diet and exercise guidelines to preserve bone during weight loss. By understanding whether the response is suppressed by caloric restriction, we can devise strategies to overcome this suppression in an acute setting and, if applied repeatedly, attenuate the loss of bone mineral density.

To maximize the potency of the anabolic response to resistance exercise, dietary protein is often manipulated in concert with resistance exercise. Six months of twice daily protein supplementation in combination with resistance training has been reported to increase IGF-1 concentrations at energy balance (Ballard et al. 2005). During caloric restriction, a high-protein diet in combination with resistance training has been shown to preserve muscle protein synthesis rates nearer to those observed at energy balance compared to a low-protein diet (Hector et al. 2018) and preserve, or even accrue, lean mass (Longland et al. 2016). Supplementation of whey protein after a bout of resistance exercise has been shown to elevate muscle protein synthesis above resting levels at energy balance (Areta et al. 2014), while resistance training alone has been shown to match, but not exceed, those observed at energy balance (Murphy et al. 2015).

Thus, to inform the development of strategies for maximizing the anabolic response to a bout of resistance exercise during caloric restriction, we first sought to measure the impact of short-term caloric restriction on the anabolic response to a bout of resistance exercise. Additionally, we quantified the impact of a single resistance exercise bout under conditions of caloric restriction on markers of bone turnover, namely sclerostin and N-terminal propeptide of type-1 collagen (P1NP), which has been shown to correlate with IGF-1 (Niemann et al. 2013). Finally, we wanted to test the impact of post-exercise protein supplementation on the anabolic response to resistance exercise in the calorie-restricted state. We hypothesized that GH would be significantly elevated and IGF-1 would be significantly suppressed following resistance exercise in the calorie-restricted state compared to energy balance, indicating the development of anabolic resistance. We further hypothesized that a bout of resistance exercise would elevate bone formation, measured through P1NP, and reduce sclerostin, a measure of anti-bone formation, even under caloric restriction. Finally, we hypothesized that post-exercise protein supplementation would attenuate the suppression of IGF-1 following a bout of resistance exercise in the calorie-restricted state.

Methods

Study design

The present randomized, single-blind repeated-measures crossover trial consisted of three 3-day conditions. Two conditions restricted energy intake to 15 kcal kg FFM^{-1} (CR), while the third provided 40 kcal kg FFM^{-1}, operationally defined as the control condition (CON). These

levels of energy availability have been previously shown to induce weight loss and maintain weight, respectively, during a similar short-term intervention (Koehler et al. 2016). All conditions provided participants 1.2 g kg body weight $(BW)^{-1}$ protein, which has previously been shown to maintain lean mass in combination with resistance training during caloric restriction (Longland et al. 2016). Following a resistance exercise bout on day 3 of each condition, participants consumed a post-exercise protein beverage during one CR condition or a post-exercise carbohydrate beverage during the other CR condition and CON. Participants underwent conditions in a random order and completed a washout period of at least 2 weeks between conditions during which they resumed habitual exercise and dietary practices. With one exception, all participants completed all conditions within 8 weeks of the same school semester. The study was approved by the University of Nebraska—Lincoln's Institutional Review Board and registered at www.clinicaltrials.gov (NCT03600311).

Participants

Participants were recruited from campus and other local recreation sites via flyers, emails to campus sports clubs, and social media posts between August 1st, 2018 and May 1st, 2019. Participants were height- and weight-stable (< 0.25 inches and < 2.5 kg change in last 6 months) men and women between 19 and 30 years old with a lean body fat percentage (< 20% men, < 30% women) for their age (Borrud et al. 2010). Participants were currently active recreational weightlifters with at least 3 years of resistance training experience, which we assessed with an online questionnaire. We selected young, lean, trained participants for their larger anabolic response to exercise (Häkkinen et al. 1998; Thomas et al. 2011; Rubin et al. 2005). Young participants also have larger anabolic responses to protein intake (Moore et al. 2015) compared to older adults and lean participants lose greater amounts of lean mass during weight loss (Forbes 2000). All of these factors served to maximize our effect sizes. Recruiting trained participants ensured that participants would be able to safely complete a high-intensity bout of resistance exercise under fasted, calorie-restricted conditions. Compliance to these inclusion criteria was confirmed during an initial screening visit to the laboratory after the informed consent was signed.

Preliminary testing

During the preliminary testing, participants had their height and weight taken by an electronic stadiometer (SECA, Germany) and their body composition was estimated by bioimpedance analysis (BIA; Quadscan 4000, BodyStat, UK). Each participant performed a familiarization session in the power rack used for the barbell back squat exercise during each condition. Briefly, participants were first provided with the option of performing a self-selected warm-up from available equipment, including a treadmill, cycle ergometer, and foam roller. Participants then completed between 2 and 5 warm-up sets of the barbell back squat exercise. Once participants indicated that they were warmed-up, they selected a weight with which they knew they could complete five repetitions. Following the set, participants provided the number of repetitions in reserve (RIR) they felt they had on the previous set. Participants then increased the weight and attempted another set until they indicated ≤ 1 RIR or failed to complete five repetitions. All participants satisfied one of these criteria within three working sets. Rest intervals between sets were not controlled during preliminary testing.

Diet preparation

Participants were provided all food consumed during each 3-day condition. Diets consisted of an individually tailored combination of clinical products (Ensure Plus; 4.57 g protein · 100 $kcal^{-1}$ and Ensure High Protein; 10 g protein · 100 $kcal^{-1}$, Abbott Nutrition, USA), maltodextrin (Tate & Lyle, UK), and whey protein isolate (Isopure, USA). Participants were allowed to consume their meals in 3–4 sittings throughout the day and were asked to record their meal timings. Blinding was achieved by matching the total volume between conditions via dilutions with water. During the conditions, participants were permitted to consume water ad libitum, but no other products.

Following their resistance exercise bout on day 3, participants received isocaloric post-exercise beverages consisting of 30 g whey protein isolate [CR with protein (CRP)] or maltodextrin [CON and CR with carbohydrate (CRC)] dissolved in 400 mL water. These beverages were consumed in addition to the provided 15 or 40 kcal · kg FFM^{-1} and 1.2 g · kg BW^{-1} protein. Participants were blinded to which beverage they received through a flavored water enhancer. Meals on day 3 were consumed at standardized times relative to blood draws to minimize interference with the exercise response (Fig. 1).

Supplementation

To mitigate differences in calcium and vitamin D consumption, we supplemented participant intake of these micronutrients throughout the entire study, including washout periods. Calcium and vitamin D provided by each condition were supplemented to make up the difference from the maximal amount provided during one condition. Supplementation of calcium during washout periods was calculated by subtracting habitual calcium intake from the maximal value provided by any condition. Habitual calcium intake through

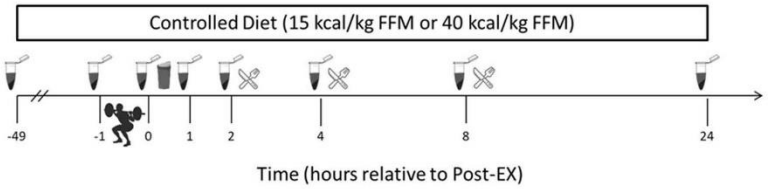

Fig. 1 Timeline of blood draws, resistance exercise bout, post-exercise protein or carbohydrate supplementation and day-3 meals during each 3-day condition

the diet was determined using the Brief Calcium Assessment Tool (Yang et al. 2010). Vitamin D was supplemented at the maximal amount provided by any condition. Participants were provided all supplements in pill boxes spacing them into 1–3 doses per day depending on number of supplements needing to be consumed.

Resistance exercise bout

On day 3 of each condition, participants reported to the laboratory between 0700 and 0900 h following an overnight fast (≥ 10 h) to perform five sets of five repetitions (5 × 5) of the barbell back squat exercise. All visits for a participant occurred within 0.5 h of the same time each morning. Participants utilized the same warm-up procedures from their preliminary testing visit before beginning their first set of the 5 × 5 with the heaviest weight at which they successfully completed five repetitions with at least 1 RIR during the preliminary testing visit. After the first set, participants provided their RIR and the weight was adjusted according to a standardized system. Participants who indicated 0 RIR or did not complete their set decreased the weight on the bar. When participants indicated 1 or 2 RIR, the weight on the bar stayed the same in the next set. If participants indicated 3 or more RIR, the weight on the bar increased for the next set. Between working sets, participants were required to rest for at least 2 min and could not rest longer than 5 min. A large rest range was permitted to ensure that participants were able to recover between sets in the manner which they habitually trained.

Participants were not allowed to exercise 24 h prior to or during each 3-day condition outside of their 5 × 5 exercise bout. Strenuous physical activity was also discouraged. Compliance with these procedures was assessed via a waist-worn accelerometer (ActiLife G3TX +, ActiGraph, USA).

Body weight and composition

Before and after each 3-day condition, participants reported to the laboratory following an overnight fast of at least 10 h where body weight was measured and body composition was assessed via Dual-Energy X-ray Absorptiometry scans (iDXA, GE Healthcare, USA). We assessed hydration status by measuring the specific gravity of each morning urine (Armstrong et al. 2010).

Blood collection and serum assays

Assays were performed on fasted blood samples collected in the morning of days 1, 3, and 4 of each condition. Additional samples were obtained serially 0-, 1-, 2-, 4-, and 8-h post-exercise on day 3 of each condition (Fig. 1). All blood samples collected throughout the study were stored as serum aliquots at − 80 °C until analysis. Commercially available assays were used to measure serum concentrations of IGF-1 [R&D Systems], GH [R&D Systems], P1NP [ABClonal], and sclerostin [Biomedica]. Our intraassay variabilities for each assay were 2.44% (IGF-1, sensitivity: 0.056 ng/mL), 4.82% (GH, sensitivity: 7.18 pg/mL), 9.23% (P1NP, sensitivity: 0.91 ng/mL), and 8.31% (sclerostin, sensitivity: 72 pg/mL).

Calculations

Prior to data analysis, data were examined for outliers, which were removed from the data set prior to proceeding with analysis. Missing body composition data from one CR condition scan in one participant as a result of a machine malfunction were imputed using the participants' other CR condition. This decision was made due to the similarity in pre-condition mass and compartment mass between the two CR conditions (< 0.1 kg difference for all measurements) and the assumption that weight loss, and the composition of weight loss, would not differ between the two isocaloric CR conditions. To minimize the impact of sex differences in GH secretion (Luk et al. 2015), we normalized all time points of GH collection to the peak in the CON condition for each participant. Area under the curve (AUC) was calculated for GH as the area above 0 using the trapezoidal method. AUC for IGF-1 was calculated as the area below the day 3 Pre-Exercise blood draw in the same manner. Volume of exercise bouts was calculated as the product of weight lifted in kg relative to body weight in kg times the number of reps completed at that weight. Changes between time points were expressed in the original units for body composition and percentages for IGF-1 and markers of bone turnover. Concentrations from serial time points were reported in the

original units for IGF-1 due to the similarity in the initial concentrations.

Statistical analyses

We first used one-sided t tests to determine if changes in hormone concentrations or body composition between time points in each condition were significantly different from 0 in hypothesized directions. If changes were significantly different from 0 and inspection of the data suggested that group differences may exist, planned pairwise comparisons, a type of contrast, were used to test for group differences between CR and CON or CRC and CRP. To test whether the anabolic response to resistance exercise was altered during CR compared to energy balance, we applied a contrast to compare the AUC responses of GH and IGF-1 between CR and CON. To test whether resistance exercise was able to rescue changes in markers of bone turnover in CR, we compared the changes observed between day 1 and day 3 against those seen between day 1 and day 4 in each condition. Finally, to test whether post-exercise protein supplementation could rescue the blunted IGF-1 response to exercise, we applied a contrast to compare the AUC responses of IGF-1 between CRC and CRP. Differences in urine-specific gravity were assessed with an omnibus F test. Additionally, we reported Cohen's d, or the difference in group means divided by the pooled standard deviation, as effect sizes (Cohen 1988). Sample size was determined based on the literature reporting changes in IGF-1 following the reduction in energy availability to 10 kcal kg FFM^{-1} day^{-1} or 20 kcal kg FFM^{-1} day^{-1} for 5 days (Loucks and Thuma 2003). Based on these data, the expected d was between 1.2 and 1.5, and a sample size of $n=6$ was sufficient to detect between-group differences of 1.2 with a power of 0.80. All statistical analysis was performed using R (R Core Team, Version 3.6). Unless otherwise stated, all data in text and figures are reported as mean ± standard error of the mean. We defined statistical significance as $p<0.05$.

Results

Participant characteristics and compliance

Of the 15 participants who started an intervention, ten completed at least one condition and eight participants completed all three conditions. One participant was excluded retrospectively due to noncompliance with study procedures [Figure S1]. At baseline, the seven participants (five men and two women) included in the present analysis were 22 ± 2 years of age and weighed 79.4 ± 7.3 kg with 18.5 ± 2.7% body fat. They had 6 ± 1 years of resistance training experience and successfully completed five repetitions at 1.4 ± 0.1 times their body weight during preliminary testing.

Changes in body weight and composition

Participants lost weight in both CR conditions (CRP −1.9 ± 0.2 kg; CRC −1.9 ± 0.1 kg, both $p<0.001$) and in CON (−0.8 ± 0.3 kg, $p<0.01$), although weight loss in CR conditions was greater than in CON ($d=1.88$, $p<0.01$). In both CR conditions, participants lost significant fat mass (CRP −0.5 ± 0.1 kg, $p<0.01$; CRC −0.6 ± 0.2 kg, $p<0.001$) and lean mass (CRP −1.3 ± 0.3 kg, $p<0.001$; CRC −1.4 ± 0.2 kg, $p=0.001$). Changes in fat mass (−0.2 ± 0.2 kg) and lean mass (−0.5 ± 0.5 kg) in CON were not significant ($p>0.10$). The differences in fat mass ($d=0.95$, $p=0.05$) and lean mass ($d=0.91$, $p=0.06$) losses between CR and CON did not achieve statistical significance. Additionally, morning urine-specific gravity did not differ between time points ($p=0.22$).

Growth hormone response to resistance exercise

All participants successfully performed 25 repetitions during the 5×5 in each condition besides one participant who performed only 24 repetitions in one condition. No characteristics of the resistance exercise bout, including warm-up volume, working set volume, total volume, proportion of working volume in total volume, or time rested differed between conditions (all $p>0.50$) [Table S1].

In response to the exercise bout, GH was elevated immediately post-exercise in all conditions (all $p<0.001$, 0 h vs Pre-Ex) (Fig. 2a) and returned to pre-exercise concentrations within 1 h [Table S2]. GH concentrations in CRC and CRP, respectively, peaked at 2.6 ± 0.4 and 2.5 ± 0.9 times the concentrations observed in CON. Together, peak GH

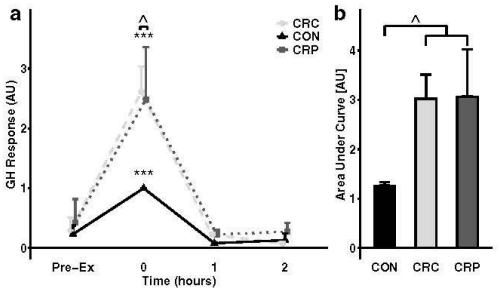

Fig. 2 Growth hormone (GH) response to resistance exercise bout after 2 days of caloric restriction (CR) or control (CON) followed by post-exercise ingestion of protein (CRP) or carbohydrate (CRC, CON). GH concentrations are normalized to the GH peak in CON ($n=6$). ***Indcates $p<0.001$ vs Pre-Ex ^indicates $p<0.05$ vs CON

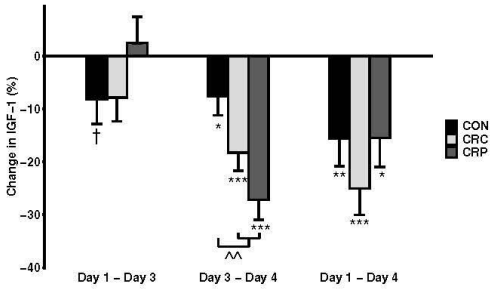

Fig. 3 Changes in IGF1 by condition and between time points, adjusted to control for order effects ($n=7$). †Indicates $p<0.10$; *indicates $p<0.05$; ** indicates $p<0.01$; ***indicates $p<0.001$; ^^indicates $p<0.01$ vs CON

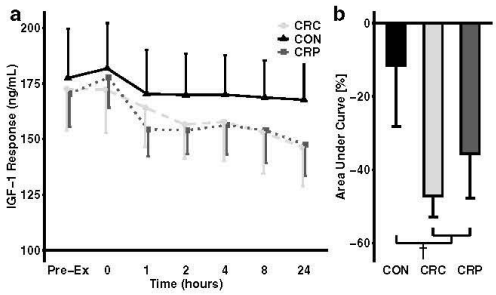

Fig. 4 Insulin-like Growth Factor-1 (IGF-1) response to resistance exercise bout after 2 days of caloric restriction (CR) or control (CON) followed by post-exercise ingestion of protein (CRP) or carbohydrate (CRC, CON) ($n=7$). †Indicates $p<0.10$ vs CON

concentrations during the two CR conditions were greater than peak concentrations during CON ($d=1.17$, $p<0.05$), resulting in a greater AUC response in the two CR conditions compared to CON ($d=1.20$, $p<0.05$) (Fig. 2b).

IGF-1 response to diet and resistance exercise

Following 2 days of controlled diet (day 1 to day 3), IGF-1 did not decrease significantly in any condition (all $p>0.05$) (Fig. 3). However, in response to the resistance exercise bout, IGF-1 decreased in all conditions (day 3 to day 4, all $p<0.05$). The decrease in IGF-1 was significantly greater in the CR conditions than in CON ($d=1.30$, $p<0.01$). Serial blood draws show the decline in AUC for IGF-1 over the 24 h following the resistance exercise bout (Fig. 4a) which was greater in the two CR conditions compared to CON (Fig. 4b), though this difference did not achieve statistical significance ($d=0.93$, $p=0.06$). There were no observable

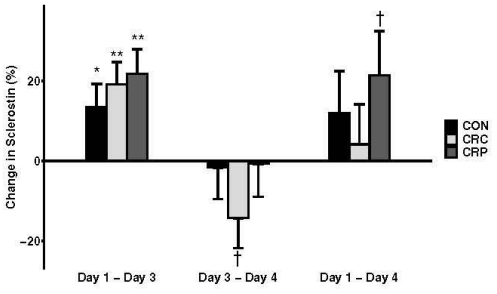

Fig. 5 Change in sclerostin by condition and between time points, adjusted to control for order effects ($n=7$). †Indicates $p<0.10$; *indicates $p<0.05$; **indicates $p<0.01$

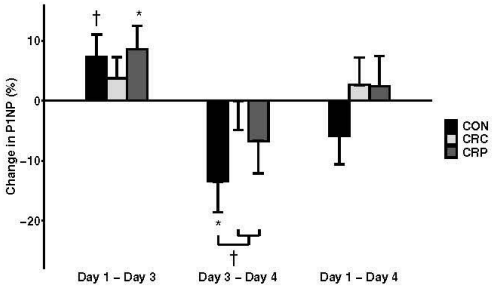

Fig. 6 Change in P1NP by condition and between time points adjusted to control for order effects ($n=7$). †Indicates $p<0.10$; *indicates $p<0.05$

differences between CRC and CRP on the IGF-1 response to resistance exercise.

Bone turnover response to diet and resistance exercise

Sclerostin increased in all conditions following 2 days on a controlled diet (day 1 to day 3, all $p<0.05$) (Fig. 5). However, following the resistance exercise bout, none of the observed elevations in sclerostin throughout each condition remained significant (day 1 to day 4, all $p>0.06$).

P1NP increased in CRP ($p=0.04$) and CON ($p=0.07$) following 2 days on a controlled diet (day 1 to day 3), although the latter did not achieve statistical significance (Fig. 6). P1NP decreased 24 h after resistance exercise (day 3 to day 4) in CON ($p=0.02$), but in neither of the CR conditions, though the difference between CON and the CR conditions did not achieve statistical significance ($d=0.80$, $p=0.06$). Overall, no significant changes in P1NP were observed across each condition as a whole (day 1 to day 4).

Discussion

The primary finding of the present intervention is that 3 days of caloric restriction at an energy availability of 15 kcal kg FFM^{-1} induced considerable anabolic resistance to a heavy resistance exercise bout. This effect occurred whilst consuming 1.2 g kg BW^{-1} protein and continued in the presence of post-exercise supplementation of either protein or carbohydrate.

We are the first to quantify the GH and IGF-1 responses to a heavy resistance exercise bout during caloric restriction. Our results show reduced IGF-1 responses 24-h following a single bout of resistance exercise despite greater peak GH concentrations immediately after the bout in the calorie-restricted state compared to the control condition. This dysregulated combination has previously been observed following exposure to low-energy availability in non-exercising populations (Loucks and Thuma 2003). However, we are the first to demonstrate this anabolic resistance induced by short-term caloric restriction persists in the presence of a potent anabolic stimulus, such as resistance exercise. We speculate that this state may reduce the potential benefits of resistance exercise to muscle mass and bone in the calorie-restricted state, but further research is needed to explore strategies for restoring the sensitivity of IGF-1 to GH stimulation and test whether outcomes such as bone mineral density or lean mass retention are improved.

We also observed a significant increase in sclerostin in response to 2 days of caloric restriction at an energy availability of 15 kcal kg FFM^{-1} without exercise. We observed an increase in sclerostin of a similar magnitude after 5 days at the same energy availability, while participants performed daily aerobic exercise and consumed a low-protein (0.8 g kg bw^{-1}) diet (Murphy et al. 2019). However, without exercise, we observed a similar magnitude of change in just 2 days despite a greater amount of protein (1.2 g kg bw^{-1}). In agreement with the previous literature, weight-bearing exercise prevented a further increase in sclerostin (Armamento-Villareal et al. 2012). In fact, the changes observed following 2 days of controlled diet without exercise were not seen across the full 3 days of any conditions, suggesting that a single resistance exercise bout may be sufficient to attenuate the elevations in sclerostin caused by short-term caloric restriction.

The changes which we observed in bone formation, measured by P1NP, were not as consistent as those observed in sclerostin. Contrary to what we hypothesized, P1NP increased over the first 2 days in one calorie-restricted condition, as well as the control condition, and decreased following the resistance exercise bout in the control condition. Resistance training has previously been shown to increase P1NP in postmenopausal women (Pasqualini et al. 2019), but we did not observe an increase 24 h after resistance exercise in this intervention. Further research is needed to replicate and confirm the influence of resistance exercise on bone markers in the calorie-restricted state.

Post-exercise supplementation of 30 g whey protein offered no discernable benefit to any outcomes reported here compared to consuming an isocaloric amount of maltodextrin. Protein feeding has been shown to provide a quicker, greater stimulus for GH release compared to carbohydrate (Pallotta and Kennedy 1968). However, in that intervention, GH concentrations peaked 2 h following protein ingestion in the absence of an exercise stimulus. In the present intervention, GH peaked immediately following the resistance exercise bout, suggesting that the resistance exercise bout may have overshadowed any potential benefit of protein ingestion by causing GH release to enter a refractory period during the time-window protein feeding may stimulate GH release. Interestingly, pre-exercise supplementation of protein has been shown to impair the GH response to a single bout of resistance exercise (Hulmi et al. 2005). Together, these results suggest that stimulation of GH by either resistance exercise or protein may suppress the ability of the other to stimulate its release by entering a refractory period. Additional research is needed to determine the interplay of exercise and protein stimulation of endocrine anabolic factors, especially during a state of caloric restriction.

The same amount of whey protein used in this intervention has previously been reported to enhance muscle protein synthesis above resting rates observed at energy balance following 5 days of caloric restriction at an energy availability of 30 kcal kg FFM^{-1} (Areta et al. 2014). However, a recent study utilizing a unique, large exercise prescription of 45 min of one-arm cranking and 8 h of walking per day for 4 days reported that skeletal muscle became immune to the anabolic effects of whey protein during an energy deficit of 5500 kcal day^{-1} (Martin-Rincon et al. 2019). Though the energy deficit targeted in that intervention exceeds our own average by more than threefold, it is plausible that whey protein may become ineffective below a threshold of energy availability and could explain why our post-exercise protein supplementation did not appear to have an impact. Additional interventions with varying levels of energy availability are needed to establish a threshold of energy availability for the benefits of whey protein.

One point of criticism about the present intervention was the inability of our control condition to maintain weight and induce a positive post-exercise IGF-1 response in all participants. However, reported post-exercise IGF-1 responses are highly variable (Kraemer et al. 2017) and the energy availability of 40 kcal kg FFM^{-1} used in this intervention has successfully maintained weight in a previous intervention

(Koehler et al. 2016). We acknowledge that different methodologies for the quantification of GH exist (Hymer et al. 2001) and the methodology employed in the present intervention may not include a comprehensive quantification of all isoforms (Hymer et al. 2019). Thus, future studies should seek to confirm our findings utilizing alternative methodologies to that in the present intervention. We further acknowledge a comprehensive quantification of the acute GH and IGF-1 responses to a bout of exercise which may benefit from additional sampling points between 0- and 1-h post-exercise; however, we observed clear differences between our control condition and the calorie-restricted conditions with the time points measured. That we still observed clear differences between the CR conditions and CON in spite of these limitations speaks to the robustness of the effects induced by our CR conditions.

The primary objective of the present intervention was to characterize the anabolic endocrine response to a bout of resistance exercise during caloric restriction. While we generally refer to this as an anabolic response, we acknowledge that there are other components of the general anabolic response, such as muscle protein synthesis (Hector et al. 2015, 2018; Murphy et al. 2015; Areta et al. 2014). However, we felt that there was a gap in the literature with regards to the endocrine response to resistance exercise under caloric restriction. While it has been questioned whether the acute IGF-1 response to exercise predicts hypertrophy during energy balance (Kraemer et al. 2017), there is evidence suggesting that signaling involved in muscle turnover downstream of IGF-1 is suppressed during caloric restriction (Martin-Rincon et al. 2019) and the suppression of IGF-1 itself during caloric restriction is linked to bone loss (De Souza and Williams 2005). This suggests that the activity of IGF-1 during caloric restriction may differ from that during energy balance. The purpose of this study was to confirm that stimulation of IGF-1 secretion by GH is impaired during caloric restriction even in the face of potent anabolic stimulation. With this framework established, subsequent studies should assess the effectiveness of countermeasures to protect against the development of anabolic resistance and, subsequently, maximize the benefits of resistance exercise in the calorie-restricted state to skeletal muscle and bone.

Conclusion

Three days of caloric restriction to an energy availability of 15 kcal kg FFM^{-1} induced considerable anabolic resistance—characterized by increased GH secretion and reduced IGF-1 secretion—to a heavy resistance exercise bout. This response occurred in the presence of post-exercise supplementation of either protein or carbohydrate. Despite this, a bout of resistance exercise did mitigate increases in sclerostin observed during each intervention. These results suggest that while resistance exercise in the calorie-restricted state can positively influence downstream tissues, such as bone, the persistence of anabolic resistance may limit the effectiveness of resistance exercise during the calorie-restricted state. Additional measures beyond post-exercise macronutrient supplementation are necessary to enhance the sensitivity of the IGF-1:GH axis to resistance exercise during caloric restriction.

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Author contribution statement CM and KK conceived and designed the research. CM conducted the experiments, analyzed data, and drafted the initial manuscript. KK revised and edited the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the University of Nebraska—Lincoln Institutional Review Board (#17933) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Study 2.3 Title: Energy Deficiency Impairs Resistance Training Gains in Lean Mass but not Strength: A Meta-Analysis and Meta-Regression

Authors: **Murphy C** and Koehler K

Abstract: Short-term energy deficits impair anabolic hormones and muscle protein synthesis. However, the effects of prolonged energy deficiency on resistance training (RT) outcomes remain unexplored. Thus, we conducted a systematic review of MEDLINE and SportDiscus for randomized controlled trials performing RT in an energy deficit (RT+ED) for ≥3 weeks. Literature was divided into studies with a parallel control group without an energy deficit (RT+CON; Analysis A) and studies without RT+CON (Analysis B). Analysis A consisted of a meta-analysis comparing gains in lean mass (LM) and strength between RT+ED and RT+CON. Studies in Analysis B were matched with separate RT+CON studies for participant and intervention characteristics, and we qualitatively compared gains in LM and strength between RT+ED and RT-CON. Finally, Analyses A and B were pooled into a meta-regression to examine the relationship between the magnitude of the energy deficit, LM and strength. Analysis A showed LM gains were impaired in RT+ED vs. RT+CON, though the effect (effect size (ES) = -0.37) did not achieve statistical significance ($p = .09$). Strength gains were comparable between conditions (ES = -0.16, $p = .49$). Analysis B supports the impairment of LM in RT+ED (ES = -0.12, $p = .02$) vs. RT+CON (ES = 0.20, $p < .001$) but not strength (RT+ED: ES = 0.87; RT+CON: ES = 0.83). Finally, the meta-regression demonstrated that an energy deficit of ~500 kcal $\cdot$ day^{-1} fully prevented gains in LM. Thus, individuals who perform RT with the goal of gaining LM should avoid prolonged energy deficiency.

Contributions: I conceptualized and designed this meta-analysis, conducted the systematic search and acquired the data. All data analyses were performed under the advisement of Drs. J. Marc Goodrich and Michael Hebert, two acknowledged professors from the University of Nebraska – Lincoln in whose class this project began. I wrote the original manuscript and revised it with the assistance of Prof. Dr. Karsten Koehler.

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ORIGINAL ARTICLE

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Energy deficiency impairs resistance training gains in lean mass but not strength: A meta-analysis and meta-regression

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Short-term energy deficits impair anabolic hormones and muscle protein synthesis. However, the effects of prolonged energy deficits on resistance training (RT) outcomes remain unexplored. Thus, we conducted a systematic review of PubMed and SportDiscus for randomized controlled trials performing RT in an energy deficit (RT+ED) for ≥3 weeks. We first divided the literature into studies with a parallel control group without an energy deficit (RT+CON; Analysis A) and studies without RT+CON (Analysis B). Analysis A consisted of a meta-analysis comparing gains in lean mass (LM) and strength between RT+ED and RT+CON. Studies in Analysis B were matched with separate RT+CON studies for participant and intervention characteristics, and we qualitatively compared the gains in LM and strength between RT+ED and RT+CON. Finally, Analyses A and B were pooled into a meta-regression examining the relationship between the magnitude of the energy deficit and LM. Analysis A showed LM gains were impaired in RT+ED vs RT+CON (effect size (ES) = −0.57, $p = 0.02$), but strength gains were comparable between conditions (ES = −0.31, $p = 0.28$). Analysis B supports the impairment of LM in RT+ED (ES: −0.11, $p = 0.03$) vs RT+CON (ES: 0.20, $p < 0.001$) but not strength (RT+ED ES: 0.84; RT+CON ES: 0.81). Finally, our meta-regression demonstrated that an energy deficit of ~500 kcal · day^{-1} prevented gains in LM. Individuals performing RT to build LM should avoid prolonged energy deficiency, and individuals performing RT to preserve LM during weight loss should avoid energy deficits >500 kcal day^{-1}.

KEYWORDS
body composition, caloric restriction, low energy availability, strength training, weightlifting

1 | INTRODUCTION

Periods of energy deficiency occur throughout the lifespan, from younger athletes within the relative energy deficiency in sport[1] or the female athlete triad[2] frameworks to older adults engaging in weight loss. Within these populations are a growing recognition that energy deficiency suppresses reproductive and metabolic hormones[3] leading to adverse health outcomes such as impaired bone health.[4,5] Despite a growing recognition of these important implications, limited knowledge of the training responses in an energy deficient state exists, particularly with respect to

resistance training (RT). RT is recommended for adults of all ages to build lean mass (LM), promote skeletal health, and improve quality of life.[6] However, adequate nutrient status is a limiting factor for the production of anabolic hormones such as insulin-like growth factor-1 (IGF-1),[7] suggesting that performing RT in an energy deficit may compromise the hormonal response to RT. Indeed, we have previously demonstrated both IGF-1 and growth hormone exhibit impaired responses to resistance exercise after as little as three days in an energy deficit.[8] Growth hormone regulates a number of metabolic processes, with which IGF-1 assists, including protein metabolism.[9] Furthermore, muscle protein synthesis is also suppressed by an energy deficient status,[10] an impairment often accompanied by the loss of LM.[11] For a more comprehensive review of the effects of low energy availability, the reader is referred to a recent review.[3]

In a field of research containing a large number of small studies, synthesis of results using methods like meta-analyses is important to objectively evaluate the effectiveness of these interventions and provide strong evidence of directions for future research. However, to our knowledge, the impact of energy deficiency on RT outcomes has never been assessed systematically in the literature. Thus, the overall objective of this meta-analysis was to test whether, and to what degree, the presence of energy deficiency attained via a reduction in dietary energy intake, attenuates training responses induced by RT. The primary aim was to quantify the discrepancy in LM accretion between interventions prescribing RT in an energy deficit (RT+ED) and interventions prescribing RT without an energy deficit (RT+CON). Our second aim was to quantify whether energy deficiency impairs strength gains in response to RT. Finally, we analyzed the impact of several moderator variables such as participant age, sex, weight status, and study duration on these outcomes. We hypothesized that LM gains, but not strength gains, would be significantly attenuated in interventions conducted in an energy deficit compared to those without. We formed this hypothesis on the basis that increases in LM are typically preceded by improvements in strength due to the earlier involvement of neuronal mechanisms compared to morphological changes.[12]

2 | METHODS

2.1 | Study design

Before beginning the systematic search process, an apparent gap in the literature was identified a priori. Based on our familiarity with the subject matter, we anticipated the number of studies employing both RT+CON and RT+ED conditions within the same intervention to be insufficient for a meta-analysis with adequate power.[13] To address this limitation, we supplemented our classical meta-analysis of studies containing both RT+CON and RT+ED conditions (Analysis A) with a qualitative comparison of separate systematic quantitative analyses of RT+CON and RT+ED studies matched for pre-defined subject and intervention characteristics (Analysis B). Finally, all studies were pooled into a meta-regression to determine the energy deficiency threshold at which LM gains are prevented.

2.2 | Inclusion criteria

For Analysis A, randomized controlled trials with at least one condition performing RT+ED and one condition performing RT+CON were included in the meta-analysis. For Analysis B, interventions needed to include only one condition performing RT+ED or RT+CON to be included. For each analysis, interventions had to contain at least three weeks of RT performed at least two times per week to align with meta-analyses on similar outcomes[14,15] and could not include concurrent aerobic training due to potential interference with both hypertrophy and strength outcomes.[16] All included studies were required to be original research and written in English.

2.3 | Search strategy

We first conducted a systematic literature search to identify potential RT+ED interventions for either Analysis A or Analysis B due to the substantially smaller body of RT+ED literature compared to RT+CON literature. This systematic literature search was conducted in PubMed and SportDiscus current to June 2021 (Supplementary Appendix 1). The original searches yielded 560 total results and two additional records were identified during the matching process described below. After screening titles, abstracts, and removing duplicates, 107 results were retained. A final count of 38 results was eligible to be included in the analysis following full-text screening (Figure 1).

After the 38 eligible RT+ED studies were identified, these were further divided into studies which contained a RT+CON group ($n = 7$), which were included in Analysis A, and studies which did not contain a RT+CON group ($n = 31$), which were eligible for Analysis B. Potential matches for the studies eligible for Analysis B were subsequently identified from a pool of literature obtained by replicating the previous search with the energy deficit terminology removed. This search yielded 24,826 results. Intervention- and population-specific terminology such as

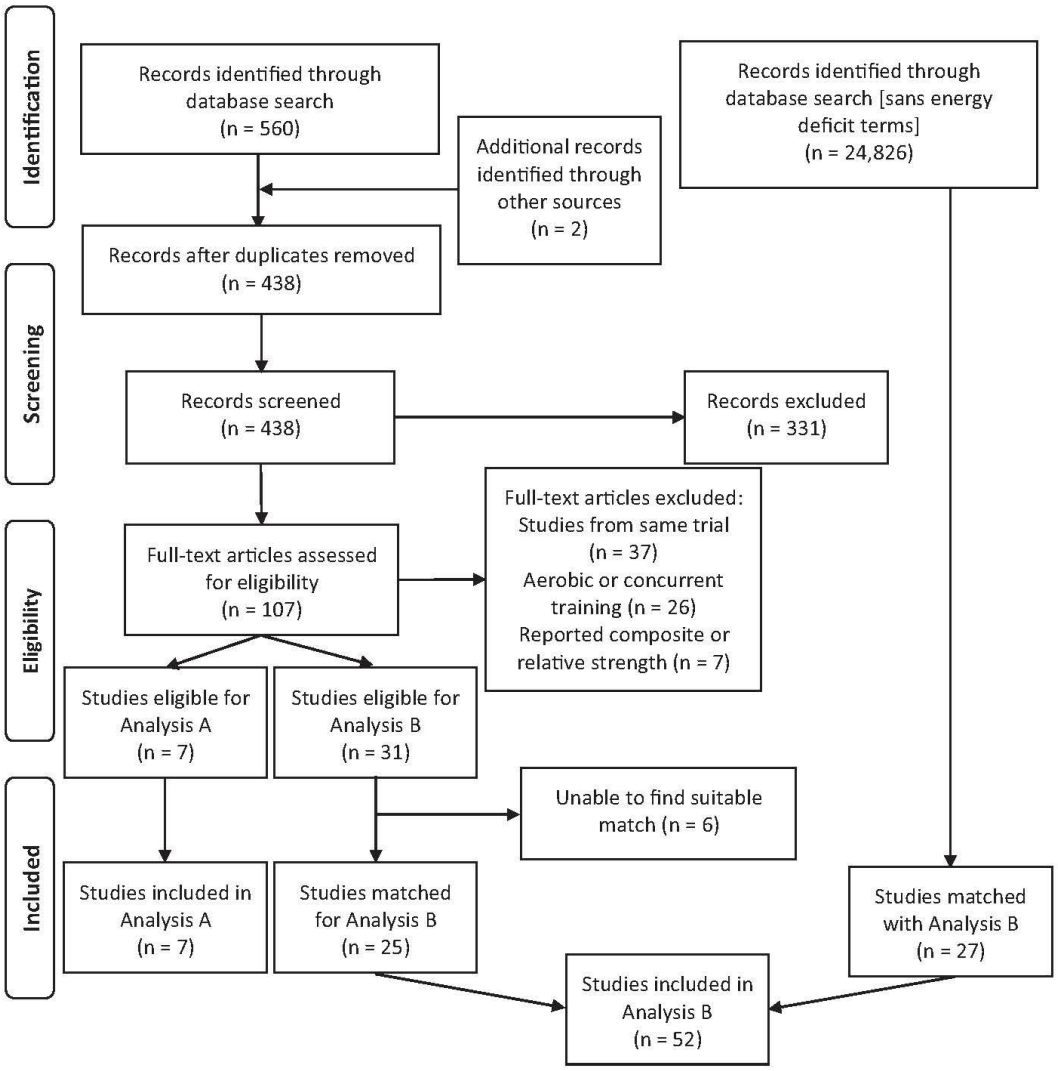

FIGURE 1 PRISMA flowchart of the systematic literature search

"postmenopausal" or "10-week" were used to identify subsets of this literature pool containing potential matches. Due to the number of sub-searches conducted, these could not be represented in Figure 1. Not all studies were able to be matched using this method. Of the original 31 results, only 25 were able to be paired and were included in Analysis B. These 25 results were paired with 27 RT+CON studies. On two occasions, one RT+ED study was paired with two RT+CON studies. In one case, a RT+ED study reporting both outcomes[17] was paired to one RT+CON study reporting LM[18] and to another RT+CON study reporting strength.[19] The other case[20] matched to two RT+CON studies for male[21] and female[22] participants separately.

In studies containing multiple RT+CON or RT+ED groups, we only included groups we could confidently match—for example, in supplement studies, placebo groups were included in the analysis over intervention groups. When macronutrient composition of the groups within a study differed, groups were matched between studies using available information to achieve a similar macronutrient distribution.

2.4 | Data extraction

Relevant variables to be extracted included pre-defined characteristics of the participants (age, sex, BMI), RT interventions (duration, frequency, sets, repetitions), and outcomes related to body composition and strength. When data were not available in text or tables, data were extracted from figures when possible using Web Plot Digitizer (V.4.2, Texas, USA: Ankit Rohatgi, 2019). Corresponding authors were solicited for information which could not be gleaned from the aforementioned sources.

Body composition outcomes extracted included LM, fat-free mass, and fat mass and had to be assessed via dual-energy X-ray absorptiometry (DXA), a preferred method for whole-body composition analysis.[23] An exception was made for one study in Analysis A using hydrostatic weighing, which has a comparable degree of accuracy with DXA on a study-wide scale.[24] However, hydrostatic weighing was not allowed for studies in Analysis B due to the high degree of variability in how the method is executed between laboratories, which could introduce unnecessary variability into the analysis. Though both LM and fat-free mass were included as primary outcomes due to data availability, the term LM will be used exclusively in this analysis to represent changes in these compartments. Per definition of the DXA methodology, the only difference between fat-free mass and LM is the inclusion of bone mass, which does not change on the same order of magnitude as LM,[25] making it a negligible factor. Thus, changes in fat-free mass and LM were considered equivalent for the present analysis. Strength was measured through either a repetition maximum strength test (e.g., one- or three-repetition maximum) or maximum voluntary contraction, but not lower intensity tests of muscular endurance due to their lower predictive reliability.[26] Strength could not be expressed relative to body weight due to the difference in weight change between groups. From the 7 studies in Analysis A, we calculated 16 body composition effect sizes from the 16 groups in 7 studies reporting body composition and 18 strength effect sizes from the 12 groups in 5 studies reporting strength. From the 52 studies in Analysis B, we calculated 44 body composition effect sizes from the 44 groups in 37 studies reporting body composition and 44 strength effect sizes from the 30 groups in 28 studies reporting strength.

2.5 | Calculation of effect sizes

All analyses were performed on effect sizes calculated as the mean change divided by the standard deviation within (SD_{within}) corrected for small sample sizes.[27] All data analysis for both Analysis A and Analysis B was conducted in R (R Core Team, Version 3.6) using the robumeta package (V.2.0, Fisher and Tipton, 2017).[28] Effect sizes are presented as means ±SD with 95% confidence intervals for all outcomes.

2.5.1 | Meta-analysis (Analysis A)

In Analysis A, the difference between pre- to post-intervention changes for RT+ED and RT+CON was used as the numerator and the denominator was calculated using the following equation where the SD for each condition refers to the SD of the change[29]:

$$SD_{within} = \sqrt{\frac{\left(\left(n_{RT+ED} - 1\right) \times SD^2_{RT+ED}\right) + \left(\left(n_{RT+CON} - 1\right) \times SD^2_{RT+CON}\right)}{n_{RT+ED} + n_{RT+CON} - 2}}.$$

In Analysis A, when SD of the change values was unavailable, they were estimated from pre- and post-intervention SD by using the following equation where r is the correlation between pre- and post-intervention measurements obtained from one representative study in the analysis for which we obtained access to complete participant data[30]:

$$SD_{change} = \sqrt{SD^2_{pre} + SD^2_{post} - \left(2 \times r \times SD_{pre} \times SD_{post}\right)}.$$

In Analysis A, effect size variance was calculated from the following formula where n_{RT+CON} and n_{RT+ED} are the sample sizes for the RT+CON and RT+ED conditions, respectively, and ES_{corr} is the effect size corrected for small sample size bias[29]:

$$V_i = \frac{\left(n_{RT+CON} + n_{RT+ED}\right)}{\left(n_{RT+CON} \times n_{RT+ED}\right)} + \frac{\left(ES^2_{corr}\right)}{2 \times \left(n_{RT+CON} + n_{RT+ED}\right)}.$$

2.5.2 | Comparative quantitative analysis (Analysis B)

In Analysis B, either the mean change or the difference between post- and pre-intervention means was used as the numerator, depending on data availability. When pre- and post-intervention SDs were available, the denominator was calculated from the following equation[29]:

$$SD_{within} = \sqrt{\frac{SD_{pre}^2 + SD_{post}^2}{2}}.$$

When pre- and post-intervention SD were unavailable, SD_{within} was calculated using the following equation where r is the correlation between pre- and post-intervention measurements. Because most of the studies did not report correlations between pre- and post-intervention measurements, an average value was calculated from the available data sets which provided this information and applied to each remaining study in the analysis[29]:

$$SD_{within} = \frac{SD_{change}}{\sqrt{2 \times (1 - r)}}.$$

In Analysis B, variance in the effect sizes was assessed using the following formula for a pre-post design meta-analysis where n is the group size, ES_{corr} is the effect size corrected for small sample bias and r is the correlation between pre- and post-measurements[29]:

$$V_i = \left(\frac{1}{n} + \frac{ES_{corr}^2}{2n}\right) \times 2(1 - r).$$

2.6 | Heterogeneity and risk of bias

Heterogeneity was reported as the I-squared value and the prediction interval derived from Tau. Risk of bias was assessed in both Analysis A and Analysis B using visual inspection of Funnel plots and accompanying Egger's Tests using the metafor package (V.2.4, Viechtbauer, 2020) for LM outcomes.[31] These analyses were not performed using strength outcomes due to the scarcity of RT papers that do not improve strength leading to false-positive risk of bias tests.

2.7 | Analysis of study characteristics

For factors on which we matched studies in Analysis B, including RT intervention characteristics and participant age, sex, and BMI, a two-tailed t test was performed to check for differences between the RT+ED and RT+CON study pools.

2.8 | Estimation of energy deficit and meta-regression

In order to assess whether outcomes were influenced not just by the presence or absence of an ED, but also by its severity, we calculated an average estimated energy deficit for each condition. Because dietary prescriptions differed between studies (e.g., consume a specific amount of kcal, reduce energy intake by a specific amount of kcal), compliance to prescriptions is generally low[32] and studies lacked sufficient information to calculate dietary intake plus all components of energy expenditure, we objectively quantified the energy deficit via changes in energy stores. To this end, the energy deficit was estimated from changes in fat mass, which was estimated to have an energy value of ~9400 kcal per kg.[33] Changes in LM were not included in the calculation to avoid autocorrelation issues, considering that LM changes are a primary outcome, as well as the difficulty of quantifying the energy cost of building LM.[34] Further, the impact of LM changes was deemed minor based on both the lack of change in the average energy deficit (<1 kcal day^{-1}) and the high correlation between the energy deficit calculated from fat mass changes and the energy deficit calculated from both fat mass and LM ($r > 0.95$) as well as the similarity between the regression outcomes with and without including changes in LM with an energy value of ~1800 kcal kg^{-1}.[35]

We first regressed our outcome variables on the estimated energy deficit. Then, to understand the contributions of other variables to the relationship between the energy deficit and our outcome variables, we assessed a group of a priori selected covariates including age,[36] weight status,[37] sex,[38] and duration of the intervention[12] because each may influence the response to RT.

3 | RESULTS

3.1 | Analysis A study characteristics

Studies included in Analysis A were published between 1988 and 2018. Analysis A contained 7 studies (6 in women exclusively, 1 in both men and women) with a total of 282 participants (60 ± 11 years) across 16 groups.[30,39-44] Only one intervention did not specify that their participants were either sedentary or physically inactive prior to the intervention.[42]

The RT interventions included in Analysis A lasted between 8 and 20 weeks (13.3 ± 4.4 weeks) and involved 2–3 sessions per week (2.9 ± 0.3 sessions) with 4–13 exercises per session (8.3 ± 2.4 exercises), 2–4 sets per exercise (2.7 ± 0.4 sets), and 8–20 repetitions per set (11.3 ± 4.1 repetitions). All included studies performed whole-body RT routines. Detailed participant and intervention characteristics for each study included in Analysis A are presented in Table S1.

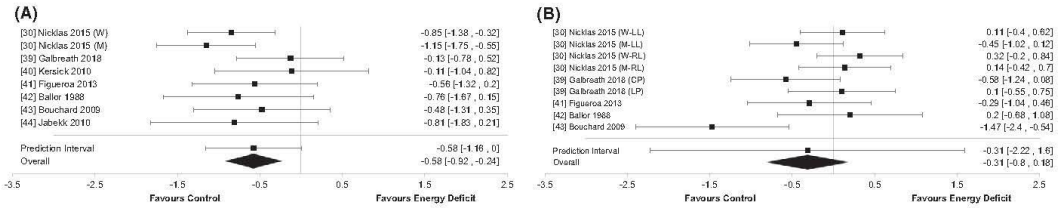

FIGURE 2 Forest plots of Analysis A for the effect on lean mass (A) and strength (B). A positive effect favors resistance training in an energy deficit while a negative effect favors resistance training without an energy deficit. Each box represents the effect size for that group and the lines around the box represent the 95% confidence interval. Abbreviations: CP, chest press; LL, left leg extension; LP, leg press; M, men; RL, right leg extension; W, women

3.2 | Analysis A: Effect of energy deficit assignment on lean mass and strength

Meta-analysis of the effect of group assignment on the relationship between RT and LM revealed a moderate effect favoring RT+CON studies over RT+ED studies (Figure 2A, effect size (ES) = −0.58, $p = 0.02$). However, there was not a significant effect of group assignment on strength (Figure 2B, ES = −0.31, $p = 0.28$). Given that only 7 and 5 studies were included in the two analyses, respectively, no moderator analyses were conducted.

3.3 | Analysis B study characteristics

Studies included in Analysis B were published between 1992 and 2018. Analysis B contained 52 studies (10 in men, 24 in women, 18 in both men and women) with a total sample size of 1213 participants (51 ± 16 years) across 57 groups.[17-22,45-90] Only one study did not specify whether their participants were either sedentary or physically inactive prior to the intervention,[81] and only one pair of studies explicitly identified their participants as resistance-trained.[55,56]

The RT interventions included in Analysis B lasted between 3 and 28 weeks (15.8 ± 6.0 weeks) and involved 2–4 sessions per week (2.9 ± 0.5 sessions) with 4–14 exercises per session (8.2 ± 2.6 exercises), 1–4 sets per exercise (2.7 ± 0.6 sets), and 1–16 repetitions per set (10.1 ± 1.9 repetitions). All included studies performed whole-body RT routines. Detailed participant and intervention characteristics for each study included in Analysis B are presented in Table S2.

In studies from Analysis B, we were successful in matching RT+ED and RT+CON groups for participant age and sex, study duration, and all RT characteristics (all $p > 0.75$). We were not, however, able to match groups for participant BMI ($p < 0.001$) due to irrevocable differences in the two bodies of literature.

3.4 | Analysis B: Qualitative comparison of changes in lean mass and strength

Figure 3 illustrates the individual group effects of RT+ED and RT+CON on LM (3A and 3B, respectively) and strength (3C and 3D, respectively). The overall effect of RT+ED on LM was negative (ES = −0.11, $p = 0.03$) while the overall effect of RT+CON on LM was positive (ES =0.20, $p < 0.001$). However, both RT+ED (ES =0.84, $p < 0.001$) and RT+CON (ES =0.81, $p < 0.001$) had large, positive effects on strength.

3.5 | Meta-regression: Estimation of energy deficit and its effect on lean mass

The pooled RT+ED groups from Analysis A and Analysis B had an average estimated energy deficit of 567 ± 350 kcal day^{-1} while the pooled RT+CON groups were in an approximate energy balance (92 ± 116 kcal day^{-1}).

Due to the apparent lack of relationship between energy deficiency and strength in Analyses A and B, we performed the meta-regression analysis only on LM. We first ran a model with no covariates regressing the change in LM on the estimated energy deficit. The intercept, representing a state of energy balance, maintained its very small, significant effect (ES =0.16, $p < 0.001$). The coefficient for the estimated energy deficit (ES = -3.1×10^{-4}, $p = 0.02$) illustrates that an energy deficit of 1000 kcal day^{-1} reduces the anticipated ES by 0.31. In other words, an energy deficit of ~500 kcal day^{-1} (ES = −0.16) would result in no LM change (ES =0; Figure 4).

We then conducted a meta-regression using the estimated energy deficit, age, sex, study duration, and BMI as predictors (Table 1). Of the variables tested, energy deficit and BMI were significant moderators, age did not achieve statistical significance as a moderator and neither sex nor study duration significantly influenced the observed

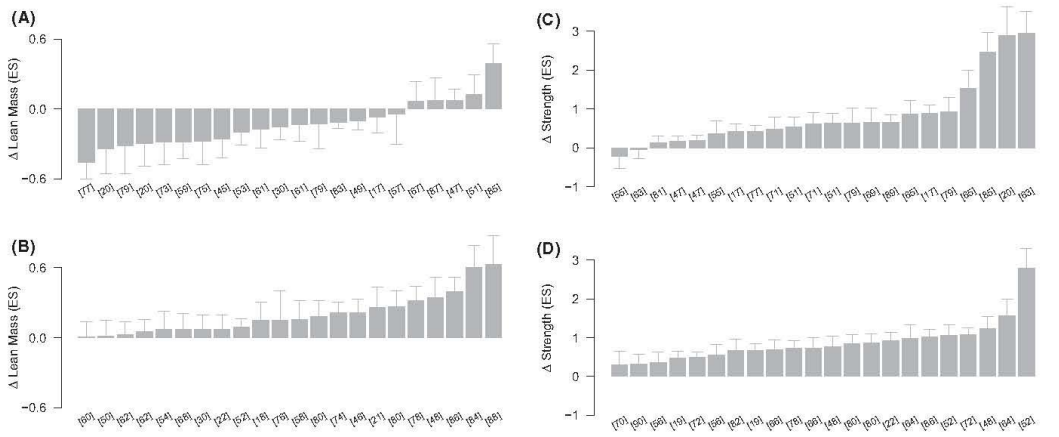

FIGURE 3 Waterfall plots of Analysis B for the effect of resistance training in an energy deficit on lean mass (A) and strength (C) and for resistance training without an energy deficit on lean mass (B) and strength (D). Numbers below the bars correspond to citation numbers where each effect was calculated. The lines around each bar represent the 95% confidence interval for the effect size

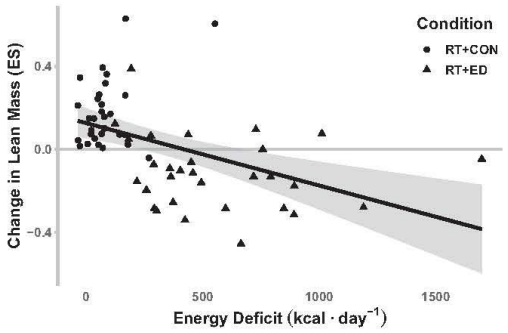

FIGURE 4 Relationship between estimated energy deficit and change in lean mass. The shaded area on either side of the regression line represents the 95% confidence interval for the regression

LM outcome. It is important to note the inclusion of covariates did not substantially alter the coefficient for the estimated energy deficit seen in the first meta-regression (ES = -3.5×10^{-4}).

3.6 | Heterogeneity and risk of bias

A substantial portion of the heterogeneity in Analysis A originated from sampling variability, in addition to between study factors ($I^2 = 0$ and 63). By contrast, a vast majority of the heterogeneity in Analysis B originated from between study factors, rather than sampling variability ($I^2 = 80$–95). Visual inspection of the Funnel Plot for LM outcomes in both Analysis A and Analysis B revealed some horizontal spread attributable to heterogeneity, but no apparent asymmetry (Figure S1). In support of this observation, the Egger's Tests (Analysis A: $z = 0.80$, $p = 0.42$; Analysis B: $z = -0.21$, $p = 0.83$) revealed no asymmetries that would suggest a publication bias.

4 | DISCUSSION

Overall, our results suggest that the presence of an energy deficit impairs the accretion of LM but not strength gains in response to RT. Furthermore, we observed that an energy deficit of 500 kcal day^{-1} (ES = −0.16) completely ablated the accretion of LM in response to RT observed in a state of energy balance (intercept ES =0.16). This result aligns with previous literature showing the commonly prescribed energy deficit of 500 kcal day^{-1} impairs LM retention.[11]

The relationship between RT and LM was influenced by the severity of the energy deficit, weight status, and age, but not sex or duration of the intervention. As a result of the regression analysis, we represented the negative association between LM gains and the strength of energy deficit as a linear relationship. However, we acknowledge the relationship between LM and energy deficit may eventually plateau, resulting in a breakpoint at which a maximal rate of LM loss occurs in the presence of RT, which may or may not be greater than the maximal rate of LM loss without the presence of RT. Despite this, the level of energy deficit required to achieve these theoretical values

TABLE 1 Meta-regression of energy deficit on lean mass effect size with all moderators

Variable	Intercept	Energy Deficit (kcal/day)	Age (years)	Sex (0 = F, 1 = M)	BMI (kg/m^2)	Study Duration (weeks)
Coefficient	1.1088	−0.0003	−0.0050	0.0668	−0.0243	0.0002
p value	0.003	0.03	0.07	0.37	0.03	0.97

was not well-represented within the included literature, if at all, due to the lack of studies with an energy deficit >1000 kcal day^{-1}. Thus, we felt both that these theoretical extremes were not of practical relevance to the research question in this population and that these data were ill-suited to explore these theoretical concepts.

Our results indicate individuals with a higher BMI gained less LM as a result of RT; however, existing literature shows lean individuals tend to lose more LM during energy-restricted weight loss.[37] Thus, RT appears to alter the relationship between body composition and composition of weight loss. It is also possible that differences in weight status between the RT+ED (BMI =32.7 ± 3.0) and RT+CON (BMI =27.5 ± 3.6) study populations may have accentuated this observed relationship.

Despite not achieving statistical significance, the negative relationship we observed between age and LM gained from RT parallels another recent meta-analysis showing a reduced impact of protein supplementation on LM with increasing age,[15] which supports the well-documented paradigm of age-related anabolic resistance.[36] Our results suggest a 500 kcal day^{-1} deficit and aging 30 years produce a similar effect on the predicted change in lean mass in response to RT (ES = −0.15). Given that energy deficiency and age influence the anabolic response to resistance exercise through the same molecular pathways[36,91] and we observed effects of each factor, the effects of energy deficiency and age appear to be additive, at least until a point of minimal response to RT.

We did not observe a significant moderation effect of sex on the relationship between RT and LM. This could be attributed to the fact that the majority of the studies included females only and that several studies conducted in both sexes failed to report the sex distribution such that they could not be used in the analysis. However, the positive coefficient of 0.07 suggests that males do add more LM than females, which is an expected observation.[38] Duration of the RT intervention was also not a significant moderator of the relationship between RT and LM. While we anticipated a positive relationship between LM gains and study duration indicating larger gains in LM from longer interventions,[12] the lack of such a relationship demonstrates significant differences in lean mass accrual within interventions 3–26 weeks in length were not detected in this analysis. This may suggest energy deficiency continues to suppress LM accretion in response to resistance exercise for as long as it is maintained; however, this hypothesis is weakened by the fact that an effect of study duration did not appear in the RT+CON studies alone (ES = −0.005, $p = 0.39$).

Strength gains were unaffected by the presence or absence of an energy deficit as well as its estimated severity. That subjects gained strength despite impaired gains, or even losses, of LM suggests these strength gains may be independent of hypertrophy and instead due to neural adaptations[12] or microarchitectural changes[92] typically preceding detectable gains in LM at the onset of a RT program. Of note, one of the two negative effects on strength in the present analysis occurred in the singular study where resistance-trained individuals trained in an energy deficit. It is unclear whether this association would be normal in experienced lifters, as not enough data exist on experienced lifters training in an energy deficit, so future research is needed to answer this question.

The covariates assessed by our meta-regression of the relationship between the severity of energy deficit and LM gained through RT did not include protein intake. While existing literature shows protein intake influences the LM gain from RT,[15] such an analysis was outside the scope of the present study for several reasons. First, while many of the included studies reported an assigned protein intake, few studies reported actual intake data. In addition, there was significant variability in how protein intake data were collected and reported which led to concerns with comparability between studies. Unlike with the severity of the energy deficit, where we were able to use changes in body composition as an objective parameter, there is no objective proxy indicator of protein intake. Thus, we felt the data were not of a high enough quality or volume to be of practical use in this analysis. Future research should emphasize accurate, objective, and homogenous reporting of dietary intake information to allow secondary analyses to be conducted accurately and efficiently.

The present meta-analysis provides statistical evidence for the observed impact of energy deficiency on the outcomes of RT, but it does not provide any mechanistic evidence. However, existing literature shows energy deficits directly impair insulin-like growth factor-1 production[7] and reduces serum concentrations in a dose-dependent

manner.[93] Whether this impaired IGF-1 production persists in the face of potent anabolic stimulation from resistance exercise has only just been investigated. We recently published a study which showed an impaired IGF-1 response following a bout of resistance exercise during three days of an energy deficit.[8] This observation combined with observed impairments in muscle protein synthesis accompanying loss of LM during energy deficiency[11] present potential mechanisms which may explain the impaired LM accretion in response to resistance exercise during caloric restriction.

While we have made substantial efforts toward ensuring an accurate and impartial meta-analysis, we recognize the present analysis has limitations. First of all, our primary analysis of studies containing both RT+CON and RT+ED groups (Analysis A) had a limited literature pool to draw from. Although we undertook a comprehensive approach to matching studies in Analysis B in order to overcome this limitation, it is impossible to create two groups as comparable as those found in randomized controlled trials when matching groups from different studies. However, we included only studies which were as comparable as possible in Analysis B by matching them on several variables including age, sex, and duration of the intervention. This resulted in only being able to match 25 of the 31 potential RT+ED studies for Analysis B. While it was originally our intention to match for weight status as well, this proved to be impossible due to irrevocable differences in the study populations between available RT+CON and RT+ED literature. Furthermore, though all studies included in the LM analysis used DXA scans, we recognize there may be differences between different machines and protocols for measurement. Despite these limitations, it is encouraging that the results of Analysis A parallel those from Analysis B.

Low energy availability is a more widely recognized perspective than energy deficiency, but we were unable to quantify energy availability within this analysis. Future research in this field should endeavor to report sufficient dietary and exercise information for the calculation of energy availability. However, our objective calculation of energy deficiency from changes in whole-body fat mass circumvented common issues such as absence of or differences in quantification of energy intake, energy expenditure, and energy requirements. By definition, an energy deficit may be induced via a reduced energy intake, increased exercise energy expenditure, or a combination of both. However, for the purposes of this meta-analysis, we focused on reductions in energy intake due to the low exercise energy expenditure of RT and to obtain a clearer picture of the impact of performing RT in an energy deficit without the potential additional interference effects of aerobic training on RT outcomes.[16]

5 | CONCLUSION

In conclusion, the results of the present analysis indicate an energy deficient state impairs LM gains as a result of RT. Furthermore, the impairment of LM gains scaled with the severity of the energy deficit. However, conducting RT in an energy deficient state did not impair strength gains. With this framework of relationships established, research can now focus on alternative RT protocols or dietary strategies to overcome the gap between RT performed in the presence and absence of an energy deficit.

6 | PERSPECTIVES

While LM is lost as a function of losing weight without intervention, RT during an energy deficit is recommended to preserve LM to aid in the prevention of weight regain and improve performance. We found that performing RT in an energy deficit impaired gains in LM, but not strength, compared to those performing RT without an energy deficit. Furthermore, the common energy deficit of 500 kcal day^{-1} was sufficient to prevent gains in LM from RT in this population. Individuals looking to gain LM from RT should avoid prolonged energy deficits while individuals trying to lose weight should practice RT and maintain an energy deficit ≤500 kcal day^{-1} to maintain LM.

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AUTHOR CONTRIBUTIONS

CM developed and conducted the systematic search and acquired the data. CM performed the data analysis with guidance from JMG and MH. All authors contributed to the conceptualization and design of the study, assisted with the interpretation, wrote and revised the manuscript, and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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3 Summary

This dissertation aimed to characterize the impact of an energy deficient state on muscle and bone and explore the roles of dietary protein and resistance exercise in attenuating those effects.

In the first work of the dissertation (2.1), the energy deficit created by a combination of aerobic exercise and energy restriction shifted bone turnover in favor of bone resorption and significantly reduced circulating leptin, but not IGF-1. For the first time, a high-protein diet was employed to attenuate the deleterious effects of this low energy availability (15 kcal · kg fat-free mass^{-1}) scenario. While the high-protein diet shifted the composition of weight loss towards a greater proportion of fat mass (mean difference = 0.22 kg), this was not accompanied by a significant attenuation of the decrease in IGF-1 nor any of the tested markers of bone turnover. Thus, five days of a high-protein diet was unable to significantly alter the response of markers of bone turnover nor upstream metabolic hormones to five days of energy deficiency created by a combination of aerobic exercise and energy restriction.

The next intervention (2.2) first built upon existing literature showing baseline levels of GH increased while IGF-1 decreased during energy deficiency (Loucks & Thuma, 2003), indicating impaired signaling. In this trial, for the first time, the GH : IGF-1 signaling response to a bout of resistance exercise was reported to be impaired during energy deficiency. This demonstrated that the suppressive effects of energy deficiency on IGF-1 could not be overcome by the potent anabolic stimulus of a bout of resistance exercise. Then, the ability of post-exercise protein supplementation to rescue this response was tested. Though a high-protein diet had a lower-than-expected impact on the outcomes reported in the previous trial (2.1), the use of resistance training, rather than aerobic training, may provide a better synergistic stimulus to maximize the benefits of increased dietary protein during energy deficiency. However, we did not observe any benefits of post-exercise protein supplementation on the response of the GH:IGF-1 axis or markers of bone formation in the present trial. Hence, energy deficiency dysregulates the relationship between GH and IGF-1 and this relationship is unable to be restored by a bout of resistance exercise nor improved by post-exercise protein supplementation.

Upon observing a suppression of the anabolic hormone response to resistance exercise during energy deficiency, the next step was to explore whether this dysfunctional hormonal response

translated into impaired resistance training outcomes as a result of energy deficiency. To date, resistance exercise interventions during energy deficiency are almost exclusively compared with interventions inducing an energy deficit via a reduction in energy intake alone or in combination with aerobic exercise. While establishing the beneficial effects of resistance training for individuals in an energy deficit was, of course, important, it is limiting to not consider the effects of energy deficiency on the training response. This gap in the literature created the systematic review and meta-analysis used to answer this follow-up question (2.3). Herein, interventions performing resistance exercise with and without an accompanying prescribed energy deficit (e.g., reduction in energy intake) were compared for the changes in lean mass and strength they produced. The results of this analysis indicated that energy deficiency impaired the accumulation of lean mass due to resistance training. Additionally, the degree of energy deficiency was linearly related to the change in lean mass with an intercept of ~500 kcal · day^{-1} indicating no change in lean mass. In other words, resistance training was, on average, able to increase lean mass in individuals in an energy deficit < 500 kcal · day^{-1}, but an energy deficit > 500 kcal · day^{-1} led to a loss of lean mass despite resistance training. Despite the clear negative relationship between energy deficiency and lean mass gains, no relationship between energy deficiency and strength gains from resistance training was observed. Thus, it seems the impaired GH:IGF-1 response to a bout of resistance exercise in an energy deficit observed in study 2.2 may be related to the impaired accretion of lean mass seen in study 2.3.

In this dissertation, the roles of dietary protein and resistance exercise in attenuating the adverse outcomes of energy deficiency were explored. Despite some promising literature supporting the consumption of high-protein diets during periods of energy deficiency, the protein interventions in the present work were largely unsuccessful in producing meaningful changes in the tested outcomes. This relationship between protein and the effects of energy deficiency will be discussed further in 4.2. Similarly, resistance training has been shown to benefit individuals in an energy deficit, but the present work shows the suppressive effects of an energy deficient state influence both the acute and long-term outcomes of resistance training.

4 Outlook

In this section, future directions for research raised by this dissertation are presented. Some of these questions arose directly from the results of the presented work (4.1 – 4.3) while others relate to elements of the dissertation, but not directly to the dissertation itself (4.4).

4.1 Effect of energy deficiency on relationship between resistance training and bone

"Do the long-term effects of energy deficiency on the response of bone to resistance training mirror the response of lean mass to resistance training?"

In study 2.3, performing resistance training without a prescribed energy deficit resulted in, on average, a positive change in lean mass. Meanwhile, performing resistance training in an energy deficit, on average, resulted in a loss of lean mass. Originally, one of the aims of study 2.3 was to answer whether energy deficiency similarly impaired the effects of resistance training on bone mineral density. However, there were insufficient data reported in the included studies to perform an analysis despite many of the included studies assessing body composition with dual-energy x-ray absorptiometry. In the future, bone mineral density should be reported in all long-term weight loss studies assessing body composition with a methodology providing that information. In terms of evidence for whether similarities would be expected between bone mineral density and lean mass, muscle protein synthesis at rest in an energy sufficient state has been shown to equal muscle protein synthesis following stimulation by resistance exercise in an energy deficit (Hector et al., 2018). Although not a perfect comparison, monitoring changes in markers of bone turnover may provide some indication of the shift in bone metabolism prior to changes in bone mineral density similar to measuring changes in muscle protein synthesis ahead of measurable changes in skeletal muscle mass. However, no such interventions have been conducted reporting changes in bone turnover markers in response to resistance training with and without an energy deficit. As in study 2.1, markers of bone formation and resorption have been shown to decrease and increase, respectively, in a number of short-term energy deficit interventions, indicating a shift towards a more catabolic state (Ihle & Loucks, 2004; Papageorgiou et al., 2017; Zanker & Swaine, 2000). However, none of these interventions included resistance training so whether the effects on bone parallel those seen in lean mass cannot be answered through either long-term or short-term studies at the present time.

4.2 Improving resistance training outcomes in an energy deficit with high-protein diet

"Does consumption of a high-protein diet close the gap between outcomes resulting from resistance training in an energy deficit compared to resistance training at energy balance?"

The ability of a high-protein diet to close the gap between resistance training with and without an energy deficit (ES > 0.30) was originally intended to be addressed within study 2.3. However, as a result of insufficient data of consistent reporting quality, this was not possible. An existing meta-analysis has shown protein supplementation augments gains in strength and lean mass from resistance training in an energy sufficient state (Morton et al., 2018). A high-protein diet of 2.4 g · kg body weight^{-1} · day^{-1} has also been shown to augment lean mass improvements from four weeks of resistance training compared to 1.2 g · kg body weight^{-1} · day^{-1} at an energy availability of 33 kcal · kg fat-free mass^{-1} · day^{-1} (Longland et al., 2016). Compared to study 2.2 where a single bolus of post-exercise protein supplementation failed to improve the hormonal response following a bout of resistance exercise, the intervention by Longland et al. was performed at a higher energy availability (33 vs 15 kcal · kg fat-free mass^{-1} · day^{-1}) and provided a greater protein intake in their intervention group (2.4 vs 1.2 g · kg body weight^{-1} · day^{-1}). Consumption of the 30g bolus of post-exercise protein in study 2.2 only increased protein intake to ~1.6 g · kg body weight^{-1} · day^{-1}. However, 30g of post-exercise protein has been shown to augment the muscle protein synthesis response to a bout of resistance exercise at an energy availability of 30 kcal · kg fat-free mass^{-1} · day^{-1} (Areta et al., 2014). Thus, the commonality between the two successful protein interventions is the greater energy availability. As reported in study 2.3, the level of energy deficit was linearly related to the change in lean mass as a result of resistance training. Thus, it is possible that a threshold of energy availability exists between 15 and 30 kcal · kg fat-free mass^{-1} · day^{-1} below which the stimulatory effects of protein are impaired. An intervention with two levels of protein intake at multiple levels of energy availability would be required to resolve this question. Based on available literature, a crossover design where participants consumed a protein intake of 2.4 vs 1.2 g · kg body weight^{-1} · day^{-1} at energy availabilities of both 30 and 15 kcal · kg fat-free mass^{-1} · day^{-1} for 3 – 5 days to measure the muscle protein synthesis and hormonal responses to a resistance exercise bout would effectively answer the question of whether the effectiveness of protein is diminished as energy availability decreases. If the effectiveness of protein is shown to be diminished in this acute, crossover study, a long-term training study assessing differences in lean mass and strength gains from 4 – 16 weeks of resistance training could then be performed.

4.3 Time course and progression of energy deficiency effects

"Do impairments of hormonal responses observed in response to short-term energy deficiency exposure persist over greater durations? Do the losses of bone mineral density and skeletal muscle change as a product of time spent in an energy deficient state?"

Outside of overweight and obese populations engaging in intentional weight loss to improve their health, the effects of energy deficiency are studied during short-term interventions in young participants. This is done to ensure the health risks to participants are minimized. For example, changes in bone turnover markers resulting from 3-5 days of energy deficiency have a negligible impact on bone mineral density. However, exposing participants to months or years of energy deficiency could compromise their bone mineral density, which can take years to recover (De Souza et al., 2014). When more robust outcomes need to be assessed, measurements are often taken cross-sectionally from high-risk populations to compare these outcomes between groups or as a factor of some past history (Nose-Ogura et al., 2020). This leaves a gap in the field's understanding of how the time course of chronic energy deficits, particularly in an energy availability context, plays out. The Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) phase II intervention measured the impacts of reducing energy intake for two years at 6, 12 and 24 months. The researchers designed the intervention to induce weight loss over the first year and maintain weight for an additional year. However, despite weight regain over the second year, which indicates a positive energy balance, bone mineral density continued to decline (Villareal et al., 2016). This suggests that the reduced energy availability decreased bone mineral density despite a positive energy balance. This dichotomy provides evidence that weight loss (energy balance) literature may not be appropriate to infer the effects of low energy availability and highlights the need for long-term low energy availability studies. One way to study the long-term effects of low energy availability with a longitudinal component would be to perform an observational study in high-risk athletic populations, such as collegiate track and field or gymnastics teams. The demand for monitoring and testing of collegiate athletes is growing and provides potential opportunities for many exercise science departments to observe medium- (in the first semester on campus) and long-term effects (throughout the athletes' careers at the university). Observational studies such as these could reveal critical time periods around which interventions can be designed.

4.4 Energy deficiency or aerobic training per se—interference with resistance training

"Are interference effects—whereby concurrent aerobic training impairs resistance training outcomes—solely the product of an energy deficient state created by the energy expenditure of aerobic training or does aerobic training itself interfere with said outcomes, even when an energy deficient state is not present?"

The interference effects of concurrent aerobic training on outcomes of resistance training, including lean mass, strength and power are well-established (Wilson et al., 2012). However, the relative contributions of aerobic training per se and the energy deficit created by aerobic training to the impaired resistance training outcomes have not been resolved. Unfortunately, no study to date has been conducted with the required groups to completely resolve this question. Ideally, one study would include the following interventions: (1) resistance training with a reduction in energy intake, (2) resistance training with concurrent aerobic training expending the same number of kcal as are restricted, (3) resistance training with concurrent aerobic training and refeeding of expended energy and (4) resistance training only. In this way, the separate and combined effects of aerobic training (groups 2 and 3) and an energy deficit (groups 1 and 2) could be assessed compared to a resistance training control group (groups 4). In the absence of this perfect study, existing information can be used to form a hypothesis. Comparing the results of study 2.3 to the aforementioned meta-analysis by Wilson et al. (2012) shows an energy deficit compromised lean mass but not strength gains while aerobic training compromised both lean mass and strength as well as power. This discrepancy suggests the presence of some independent effects of aerobic training on skeletal muscle adaptations to resistance training. Notably, though, there were significant negative correlations between the frequency and duration of aerobic training and the resistance training outcomes, which may suggest a contribution of the energy deficit as increasing frequency and duration both increase the energy expenditure of aerobic training. However, a combined aerobic and resistance training program did not produce significantly different changes in lean mass or strength compared to resistance training alone in a moderate energy deficit of 500 – 750 kcal $\cdot$ day^{-1} (Villareal et al., 2017). Considering the effects of refeeding on aerobic training alone, refeeding did not benefit lean mass gains as a result of aerobic training (+2.0 kg without refeeding, +1.2 kg with refeeding) in a 12-week intervention in overweight men (Nordby et al., 2015). Whether this indicates refeeding the energy expended by aerobic training would influence the lean mass gains induced by resistance training, however, can still be debated.

5 Abbreviations

GH.....growth hormone
IGF-1.....insulin-like growth factor 1
kcal.....kilocalories
RED-S.....Relative Energy Deficiency in Sport

6 Figures

7 References

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8 Appendix

8.1 Complete list of publications

Murphy C and Koehler K. “Energy Deficiency Impairs Resistance Training Gains in Lean Mass but not Strength: A Meta-Analysis and Meta-Regression.” *Scandinavian Journal of Medicine and Science in Sports,* 2021; epub ahead of print. DOI: 10.1111/sms.14075.

Murphy C, Bilek LD and Koehler K. “Low energy availability with and without a high-protein diet suppresses bone formation and increases bone resorption in men: a randomized controlled pilot study.” *Nutrients*, 2021; 13(3): 802. DOI: 10.3390/nu13030802.

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Murphy C, Takahashi S, Bovaird J and Koehler K. “Relation of aerobic fitness, eating behavior and physical activity to body composition in college-age women: a path analysis.” *Journal of American College Health*, 2019. DOI: 10.1080/07448481.2019.1647210.

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Conference Abstracts

Murphy C and Koehler K. “Caloric restriction induces anabolic resistance to resistance exercise.” *German Journal of Sports Medicine*, 2020; 71(2_supplement): S9.
[Oral Presentation at: Fitnesswissenschaftskongress 2020]

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