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Attenuating the Side Effects of Caloric Restriction Through Exercise and Increased Protein Intake

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ATTENUATING THE SIDE EFFECTS OF CALORIC RESTRICTION THROUGH EXERCISE AND INCREASED PROTEIN INTAKE

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ATTENUATING THE SIDE EFFECTS OF CALORIC RESTRICTION THROUGH EXERCISE AND INCREASED PROTEIN INTAKE

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The effects of caloric restriction (CR) on weight loss and health outcomes are documented, but few controlled studies have addressed its effect on performance. Fat free mass (FFM) is reduced during CR, which may impair performance. The purpose of this thesis was to explore the capacity of these strategies to attenuate the side-effects of calorie-restricted weight loss: Exercise, which preserves FFM during CR, may be employed to maintain performance in an energy-deficient state, and a high protein intake may work in combination with exercise to further protect FFM and performance. Two studies were utilized to address this purpose. In study 1, participants (N=6) underwent two, 4-day periods of CR (15 kcal/kg FFM/day) and two periods of balanced energy availability (40 kcal/kg FFM/day). During one CR (CR+EX) and one balanced condition (CON+EX), participants exercised to expend 15 kcal/kg FFM/day; no exercise was conducted during the other conditions. Body weight decreased in both CR+EX (-1.8kg [-2.6; -1.0]) and CR without exercise (CR-EX) (-2.4kg [-3.0; -1.9]), but FFM tended to decrease only in CR-EX ($p=0.07$). Peak aerobic capacity (VO_{2peak}) increased (6.2% [2.8; 9.5]) in CR+EX. Submaximal heart rate and rating of perceived exertion increased in CR-EX ($p<0.05$). In CR conditions, there was an increase submaximal fat oxidation (CR+EX: 129% [29; 229]; CR-EX: 126% [84; 168]). No significant changes were found in anaerobic performance, leg curl, and leg extension, but bench press maximal force

decreased in CR+EX ($p = 0.04$). In CR-EX there was a decrease in positive mood, and self-confidence. In study 2, 6 participants underwent two, 5-day periods of CR, one with a high protein diet (1.7 g/kg; CR+HP) and one with a low protein intake (0.8 g/kg; CR+LP). During an energy-balance control condition (CON), participants also consumed 1.7 g/kg protein. Exercise was conducted in all conditions. Body weight decreased in CR+HP (-3.5 kg [-4.9; -2.0]) and CR+LP (-2.4 kg [-3.0; -2.1). VO_{2peak} (+6.8 mL/kg/min [2.7; 10.8]) as well as maximal fat oxidation (+0.34 g/min [0.32; 0.36]) increased during CR+HP. Overall, our findings suggest exercise and a high protein diet seem effective in preserving performance during short-term CR.

TABLE OF CONTENTS

LIST OF MULTIMEDIA OBJECTS

Figures

Tables

CHAPTER 1: INTRODUCTION

Many individuals attempt to lose weight through caloric restriction (CR), which refers to the restriction of caloric intake with the goal of attaining an energy deficit. CR results in the loss of lean mass and a suppression of metabolic processes such as growth and reproduction, which may all work in conjunction to negatively affect many biological functions, including exercise performance and bone health [1, 2]. Exercise, which preserves lean mass during weight loss, may be a strategy to maintain exercise performance in an energy-deficient state [3]. A high dietary protein intake may work in combination with exercise to further protect lean mass, likely due to the maintenance of the anabolic sensitivity of the musculoskeletal system, and may also favor the preservation of bone [4]. The overall purpose of this thesis was to explore the capacity of these strategies to attenuate the side-effects of calorie-restricted weight loss. Two studies were utilized to address this purpose.

Study 1

Aim 1: Determine the effects of a short-term, controlled energy deficit on measures of performance and exercise metabolism

Hypothesis 1A: CR will result in a reduction of FFM, maximal aerobic capacity, submaximal aerobic performance, and perceived physical and mental state

Hypothesis 1B: CR will not result in significant changes in anaerobic performance and measures of muscle strength

Aim 2: Explore whether exercise preserves the effects of a short-term, controlled energy deficit on these same outcomes.

> *Hypothesis 2:* Exercise will attenuate reductions in FFM, maximal aerobic capacity, submaximal aerobic performance, and perceived physical and mental state.

Rationale: *Hypothesis 1A* is based on previous findings suggesting that about 25% of weight loss are typically lost as FFM during calorie-restricted weight loss [3]. In addition, previous research has aerobic performance is impaired during an energy deficit, which has been observed by reductions in submaximal aerobic performance, maximal aerobic capacity, and increased exertion during aerobic exercise during short-term interventions involving caloric restriction [5-8]. Other factors such as fatigue, stress, and depression, which are common symptoms of chronic low energy availability (EA), may also impair exercise performance [9]. *Hypothesis 1B* is based on previous findings suggesting that, unlike aerobic performance, anaerobic performance seems to be unaffected by an energy deficit, as shown by an absence of changes in anaerobic performance during short-term energy deficit interventions [10, 11]. Although anaerobic performance does not seem to be affected, the results of an energy deficit on strength measures are mixed. Reductions in isometric hand grip and back strength have been reported [12, 13], although various measures of unweighted jump height did not change and weighted jump height increased during short-term, prescribed energy deficit interventions [10, 14]. *Hypothesis 2* is based on previous findings suggesting that the implementation of exercise seems to offset some of the negative effects of CR, as shown by the restoration of muscle protein synthesis to

normal levels after a bout of exercise in an energy deficit [15]. Further, exercise has been shown to attenuate the loss of FFM during weight loss [3, 16-19]. Combining CR with exercise reduces the weight lost as FFM from 25% to 12% [9]. Exercise may further benefit exercise performance during an energy deficit. For example, middle-aged obese men and women increased their maximal aerobic capacity when exercising during a 12 week CR intervention [18]. Although exercise generally has a positive effect on mood and depression [20], it is unclear if it positively impacts mood in an energy deficit [13, 14, 21].

Study 2:

Aim 1: Determine if an increased protein intake preserves FFM and aerobic performance during an energy deficit.

> *Hypothesis 1:* When compared to a low protein diet, the consumption of a high protein diet during CR will preserve FFM and aerobic performance.

Aim 2: Determine if increased protein intake attenuates reductions of anabolic hormones (IGF-1, IGF-BP3, and insulin)

> *Hypothesis 2:* A high protein diet will attenuate reductions of the anabolic hormones IGF-1 and IGF-BP3, but not insulin.

Rationale: *Hypothesis 1* is based on studies that propose a high protein intake during an energy deficit, especially when coupled with exercise, may further offset some of the negative consequences of an energy deficit. This is suggested by an increase in muscle

protein synthesis above normal levels when protein is consumed after a bout of exercise in an energy deficit [15]. Recent research suggest that FFM may be protected when consuming a high protein diet, defined as an intake greater than the RDA of 0.8 g/kg, in an energy deficit [19, 22-26]. The maintenance of FFM when participants consume a high protein diet alludes to a high protein diet's potential to protect physical function such as aerobic performance. *Hypothesis 2* is based on recent research suggesting that high protein intake during an energy deficit may further help to maintain the anabolic sensitivity of FFM and bone. The impaired secretion of IGF-1 during a low protein intake suggests that a high protein may be able to attenuate the reduction in IGF-1 during an energy deficit in humans [27]. A lack of protein was found to impair growth hormone (GH) secretion, thus resulting in reduced secretion of IGF-1 and impairing growth in a rat model [28]. Mixed results were found regarding the effect of a high protein diet on IGF-1 during an energy deficit. [29-33]. Although few studies have explored this, high protein intake seems to not affect the suppression of insulin during an energy deficit [24] or attenuate the reduction in insulin compared to a low protein group [22] in obese individuals.

Overall, the results of these studies will inform us of the extent to which exercise and a high protein intake can mitigate some of the side effects of calorie-restricted weight loss.

CHAPTER 2: LITERATURE REVIEW

Non-adipose Tissues are also lost during Weight Loss

Weight loss requires an energy deficit, which is characterized by expending a greater amount of calories than are consumed through the diet and results in a negative energy balance [34]. Restricting calorie intake, increasing energy expenditure through exercise, or through a combination of both can be utilized to lose weight [35]. In most cases, weight loss results in not only a loss of fat mass, but also a loss of non-fat comprised tissues [35]. In an energy deficit, body protein can be utilized as additional sources of energy and is made available through increased protein catabolism, which occurs primarily in expendable tissues such as the skeletal muscle [36]. Muscle protein breakdown results in the release of amino acids which are ultimately converted into acetoacetyl-CoA, acetyl-CoA, or pyruvate and serve as intermediates for the TCA cycle or ketone body synthesis to supply additional energy for brain and vital organ function [37].

When an energy deficit is achieved through CR, typically about 25% of weight loss is typically lost as FFM in overweight and obese populations [3]. The proportion of FFM loss can be even greater in normal weight individuals due to their lower availability of fat mass that can be utilized for energy, suggesting that the loss of FFM is a function of initial body fat content [38]. The loss of FFM can result in major health detriments, including impaired functional capacity resulting from the loss of skeletal muscle mass and reduced strength [1], decreased blood glucose circulation [7, 11, 39], and reduced insulin sensitivity [2].

Implications for Exercise performance

The loss of FFM during CR may not only lead to major health detriments, but may also impair exercise performance. The results of previous studies have shown that *aerobic performance* is impaired during an energy deficit, which has been observed as reductions in submaximal aerobic performance, maximal aerobic capacity, and increased exertion during aerobic exercise when undergoing short-term interventions utilizing CR [5-8]. During a 5-day intervention of CR and prolonged exercise in young male soldiers, an 8% reduction in maximal oxygen uptake (VO_{2max}) and a 14% decrease in cycling power at VO_{2max} has been reported [7]. When testing competitive cyclists, time to fatigue was reduced by 15-22% and perceived exertion increased after a 24 hour fast [5]. CR for 48 hours in young aerobically fit males resulted in a 10% reduction in distance converted during a 30 minute treadmill time trial.

Unlike aerobic performance, *anaerobic performance* seems to be unaffected by a short-term energy deficit, as shown by an absence of changes in anaerobic performance during short-term interventions utilizing CR [10, 11]. In experienced male judo athletes, a 5% loss in bodyweight over 5 days resulted in no change in Wingate cycling performance, an anaerobic cycling test that measures peak anaerobic power and capacity [40], or number of judo attacks in a specific judo exercise [11]. Another study in wrestlers and judo athletes found no change in Wingate cycling performance or 30 m sprint time after a 5% loss in body weight over either 3 days or 3 weeks [10]. However, contrary to the effects on Wingate performance, 7 days of CR in judo athletes resulted in a reduction in 30 second jumping test and 30 second arm ergometer performance, but no change in the 7 second jump test performance or 7 second arm ergometer test

performance [14]. From this, it is clear that anaerobic performance, primarily when measured via a Wingate test, does not change during CR, although mixed results have been reported for the effect of CR on exercises utilizing the arm ergometer.

Similar to measure of anaerobic performance, studies assessing the impact of CR on *strength measures* have shown varying results. When national level judo athletes lost 5% of their body weight within a week, a 5% reduction in isometric hand grip strength occurred. Reductions in isometric hand grip as well as back strength have also been reported during short-term energy deficit interventions [12, 13]. Similarly, 7 days of CR in young wrestlers resulted in a 6% reduction in isometric hand grip strength and a 10% reduction in isometric back strength [12]. Unlike isometric strength measures, various measures of unweighted jump height either did not change during a short-term, prescribed energy deficit [10, 14]. Another study, using wrestlers and judo athletes who lost 5% of their body weight either rapidly (3 days) or gradually (3 weeks), found that weight loss did not impair unweighted vertical jump height, but increased weighted jump height by 6-8% when the weight loss was gradual [10]. From this, it is possible that a more severe energy deficit is more likely to impair the ability to increase strength, although the loss in body weight may have prevented a reduction in jump height measures. Other factors such as fatigue, stress, and depression, which are common symptoms of chronic CR, may also impair exercise performance [9]. Previously discussed interventions have revealed increases in confusion, anger, tension, and fatigue along with decreased vigor during 7 days of CR in Judo athletes [13, 14].

Bone Mass is reduced during Weight Loss

Although weight loss as a result of CR leads to the loss of fat mass and FFM, bone is another tissue impaired by CR. When energy substrate levels are low, reduced anabolic hormone levels combined with increased cortisol levels contribute to impaired calcium incorporation into bone [41]. In this state, osteoblasts are stimulated to secrete receptor activator of nuclear factor ligand (RANKL) and osteoclast differentiation inducing factors, activating and or producing osteoclasts. The osteoclasts then secrete lysosomal enzymes to break down the matrix around the osteocytes, thus demineralizing the bone [42, 43].

The increased breakdown of bone during CR may lead to a loss of bone mineral density (BMD) in the long-term. A recent meta-analysis demonstrated that weight loss resulted in significant reductions in BMD at the hip and lumbar spine in adults. The meta-analysis was conducted using studies observing the effects of weight loss on BMD in adult males and females undergoing a weight loss program where BMD of the total hip, lumbar spine, or total body was the primary outcome of interest. The hip and spine BMD were chosen since individuals who have low BMD in these areas are considered candidates for treatment for increased risk of fracture [44]. A significant effect of weight loss on hip and lumbar BMD was observed in the included studies. Over at least 4 months of CR, hip BMD decreased by 0.006 to 0.008 g/cm^2 ($p < 0.001$). CR longer than 13 months resulted in decreases in lumbar spine BMD by 0.017 to 0.019 g/cm^2 ($p < 0.001$) [45]. Increased loss of BMD was also found in a systematic review of clinical trials on effects of CR in overweight and obese adults. Weight loss caused by CR resulted in 1-1.5% (0.010 to 0.015 g/cm^2) decrease in hip BMD during interventions with a

duration of 6 months or longer, but no significant reduction in lumbar spine BMD were reported. The observed decrease in hip BMD is equivalent to a 10-15% increase in fracture risk [46]. Overall, the findings show a reduction in BMD during extended periods of CR and an increased risk of fractures.

The reduction in BMD during extended periods of CR is a result of increased bone demineralization, as indicated by characteristic changes in markers of bone formation and turnover. Serum osteocalcin is produced by osteoblasts to promote bone formation and is often used as a marker of bone formation [43]. C-terminal telopeptide of type I collagen is highly correlated to bone turnover rate because type I collagen is the part that is cleaved by osteoclast during bone resorption, indicating the activity level of the osteoclasts [47]. N-terminal telopeptide of type I collagen is also a marker of bone turnover, but is much more sensitive and fluctuates in a nonspecific manner [48]. In the systematic review by Zibellini et al., bone formation (serum osteocalcin: 0.26 nmol/L; +16.3%) and measures of bone turnover (c-terminal telopeptide of type I collagen: 4.72nmol/L; +37.8%) (n-terminal telopeptide of type I collagen: 3.70 nmol/L; +23.9%) increased during 2-3 month CR interventions, but not during interventions lasting longer than 6 months [46]. In young obese individuals undergoing CR for 6 month, hip BMD did not decline but bone alkaline phosphatase levels, a glycoprotein found on the surface of osteoblast and indicates the activity of bone formation [49], decreased by 23% in the CR group, suggesting an increase in bone turnover [50]. The findings show that an early onset of increased bone turnover occurs as a result of CR, but the effects on measures of BMD are not seen until after interventions lasting longer than 6 months.

Overall, there is much evidence suggesting that changes in bone tissues occurs as a result of CR, primarily an increase in bone resorption and decreased bone formation, which may lead to reduced bone strength and osteoporosis later in life [51].

Endocrine Pathways Involved in Weight Loss

The loss of FFM, bone as well as the suppression of metabolic functions are modulated by the suppression of key endocrine pathways in the energy deficient state. Hormones that are primarily impacted during an energy deficit include leptin, a key signal of energy state, as well as hormones with anabolic and anti-catabolic capacity, such as leptin, IGF-1, ghrelin, insulin, and sex hormones [6, 7, 11, 39, 52].

Leptin acts as a signal of metabolic state and is critically involved in the regulation of growth and reproduction. Leptin is secreted by adipocytes and circulating concentrations are directionally proportional to the amount of fat stores [53]. The main biological function of leptin is the promotion of satiety, but it also serves as a key regulator of energy balance and metabolism [54]. In the energy-deficient state, leptin is readily suppressed beyond what is expected from the loss of fat mass, which has downstream effects on many other endocrine axes [53].

IGF-1 acts to mediate the effects of GH and stimulates growth in most cells of the body [43]. In a fed state, growth hormone releasing hormone (GHRH) is released from the hypothalamus and stimulates the anterior pituitary to secrete GH. GH then stimulates the liver as well as other tissues to secrete IGF-1, which upon binding to the IGF-1 receptor simulates the AKT signaling pathway and promotes cell growth and inhibition of cell death [43]. In bone, IGF-1 stimulates the development of osteocytes, promoting bone

formation [55]. During severe energy deficiency, there is a reduction in the secretion of IGF-1, although GHRH and GH are secreted to a greater extent due to a negative feedback in response to low IGF-1 [56].

Ghrelin is a hormone involved in metabolism and its secretion is dependent on food intake. The main actions involve the stimulation of appetite and promotion of a positive energy balance [43]. When in a CR state, ghrelin secretion is increased [57].

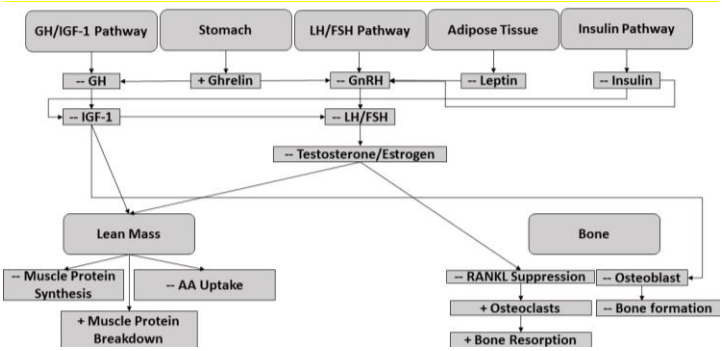

Figure 2.1: Alterations in endocrine pathways during caloric restriction that are linked to a reduced anabolic sensitivity of skeletal muscle and bone

Insulin is another metabolic hormone that is impacted by reduced energy intake. In the fed state, pancreatic beta cells secrete insulin when food is ingested to regulate blood glucose levels [54]. In addition, insulin also stimulates muscle growth via control of amino acid uptake and suppression of proteolysis [43]. Insulin further has anabolic as well as anticatabolic effects on bone through the stimulation of osteoblasts by mediation of IGF-1 signaling [58]. Insulin secretion is reduced in the energy deficient state, most likely due to reduced circulating glucose [54].

The downstream effects of alterations in these key metabolic hormones ultimately also affect the secretion of sex hormones. Beyond their primary functions of developing secondary sex characteristics and promoting reproductive function, sex hormones exert anabolic and anti-catabolic effects on the musculoskeletal system [43]. In skeletal muscle, testosterone binds to the androgen receptor and promotes cell growth [59]. In bone, testosterone is converted to estradiol, which suppresses the secretion and binding of RANKL to the osteoclasts, impairing its ability to secrete enzymes which demineralize bone [60]. Estrogen acts similarly on the suppression of bone demineralization. Multiple endocrine pathways are responsible for the suppression of sex hormones in the energy-deficient state. The suppression of leptin in the calorie-restricted state results in a reduced secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus, thereby impairing the release of LSH and FSH from the anterior pituitary and sex hormones from the gonads [54]. In addition, reductions in IGF-1 result in a reduced stimulation of GnRH from the hypothalamus [54]. Ghrelin further inhibits the secretion of sex hormones directly by suppressing the release of GnRH from the hypothalamus and indirectly by impairing the release of GH and subsequent release of IGF-1 via somatostatin [54]. Sex hormone secretion is also minimally impacted by insulin, via stimulation of GnRH release and subsequent promotion of LH and FSH secretion [54].

Consequences of Endocrine Adaptations for the Musculoskeletal System

Alterations in key metabolic hormones, including leptin, IGF-1, ghrelin, insulin, and sex hormones, in the energy deficient state result in an endocrine environment in which cell growth and development are suppressed, which may have detrimental effects

on the musculoskeletal system. The impaired secretion of IGF-1 and testosterone results in reduced skeletal muscle growth [29]. Reduced amino acid uptake and impaired suppression of proteolysis may also occur due to decreased insulin secretion [43]. The effects on skeletal muscle may not only impair muscle growth, but also lead to a loss of skeletal muscle mass secondary to the reduced secretion of IGF-1, testosterone, and insulin. As discussed previously, bone is also affected by the endocrine alterations caused by CR. The suppression of IGF-1 and insulin can result in reduced osteoblast formation and reduced stimulation of sex hormones. Impaired suppression of bone demineralization through osteoclasts also occurs as a result of the reduced circulating levels of sex hormones [43]. Consequently, suppression of IGF-1, insulin, leptin, and sex hormones is connected to a loss of bone [61] and reduced bone mineral density in the long-term [62, 63], which increases the chances of developing osteoporosis later in life [30].

Exercise Attenuates the Negative Effects of Weight Loss

Overall, the negative consequences of CR may offset the health benefits of weight loss. Therefore, there is a need for strategies that maintain the benefits of an energy deficit, such as weight loss and improved body composition, while reducing negative effects on metabolism, FFM, and bone. The implementation of exercise seems to offset some of the negative effects of CR. Fortunately, some of the benefits of exercise are able to occur in the absence of an increase in anabolic hormones, which are typically suppressed in an energy deficit [64-66].When exercise is conducted, metabolic stress also occurs when exercising and promotes muscle development. Molecular pathways promote the activation myogenic genes that promote muscle protein synthesis and impair muscle

protein breakdown, leading ultimately to a stimulation of muscle growth as a response to exercise. The anabolic stimulus of exercise minimizes the use of amino acids as an energy source and prioritizes the use of them in muscle growth and repair, resulting in a greater use of fatty acids and carbohydrates as energy sources [67, 68]. In a systematic review assessing the effects of energy restriction and exercise on FFM, weight loss in the form of FFM was reduced from 25% to only 12% of the total weight loss. The attenuated loss of FFM is shown by as little as 8% of the ED and exercise groups losing a significant amount of FFM (≥1.5 kg) versus 56% of CR without exercise groups. It should be noted that participants in the studies analyzed were older, overweight individuals who participated in interventions lasting at least 6 weeks [3]. It is clear that exercise has protective effects on FFM when undergoing CR and the mechanisms that promote hypertrophy separate of anabolic hormones may promote maintenance of FFM.

The ability of exercise to attenuate the loss of FFM during CR is not only limited to resistance exercise, but may also be effective when conducting a combination of different modes of exercise. A maintenance of lean mass and greater losses of fat mass were found in a study comparing the effects of exercise during weight loss on body composition in middle aged women. After a 4 month intervention aerobic (walking 5 days per week) and resistance exercise (2 days per week), participants who exercised during CR maintained lean mass (-1.0 kg) and lost 0.5 kg more fat mass (-5.5 kg) when compared to the non-exercising group (lean mass:-2.7 kg; fat mass: -5.0 kg) [19]. The maintenance of lean mass and a greater loss of fat mass was also reported in another 4 month CR intervention on overweight inactive women who exercised (2, 1 hr group strength/endurance training; 2, 1 hr nordic walking per week) when compared to a non-

exercising group. Lean mass was completely maintained (-0.01 kg) and a 1.4 kg greater loss of body fat (-5.1 kg) was reported in the exercising group when compared to the non-exercising group, who lost 0.8 kg of lean mass and 3.7 kg of fat mass[16]. Taken together, there is strong support for the combination of resistance and aerobic to maintain lean mass and to reduce fat mass to a greater extent during CR.

Although the combination of aerobic and resistance exercise has significant effects on muscle protein synthesis (MPS) and maintenance of lean mass, aerobic exercise alone can also help prevent the loss of fat free mass. In a study examining older overweight and obese men and women, the effects of CR and exercise on FFM were compared to the effects CR only. After a 4 month intervention of CR only or CR with moderate intensity walking (3-5 times per week for 35-45 min), participants lost similar amounts of body weight, bit unlike the CR group, no significant loss of FFM was found in the CR and exercise group [17]. Similarly, a maintenance of muscle mass was reported during a 12 week intervention in older overweight or obese men and women. Participants were assigned to either a diet or diet and aerobic exercise group where they exercised at least 300 min/wk at their lactate threshold. Both groups lost similar amounts of weight, but muscle cross-sectional area and normal-density muscle area decreased only in the diet group [18]. The findings show that aerobic exercise is also an appropriate exercise stimulus to attenuate the loss of FFM and skeletal muscle mass during weight loss.

The findings that exercise can attenuate the loss of FFM during CR are further strengthened by results of the effects of exercise on MPS. At rest, MPS rates after 5 days of an energy deficit were found to be 27% lower when compared to an energy balance, but a single bout of resistance exercise was able to restore MPS rates back to resting

levels of an energy balance [15]. Overall, there is strong evidence which suggest that exercise is able to attenuate or prevent the loss of FFM during CR regardless of the type of exercise, which may be due to the contractile activity and increased insulin sensitivity, which promotes MPS and inhibits muscle protein breakdown [69].

Exercise can also attenuate Bone Loss during Weight Loss

The anabolic effects of exercise may also have benefits with regard to maintaining bone mass during CR. Exercise provides mechanical strain to not only the muscles, but also bone, which provides a stimulus to increase bone strength and BMD. The mechanical strain can come in the form of ground reactive forces or the contractile activity of skeletal muscle, which is referred to as mechanotransduction. Osteocytes detect the mechanical stimuli and the transduction of the strain by the osteocytes to the osteoclast and osteoblasts to stimulate bone resorption and remodeling. Exercise is thought to reduce the secretion of sclerostin, which promotes anti-anabolic effects on bone formation, from the osteocyte and leads to and upregulation of osteoblastogenesis. The activity of the osteoclast and osteoblast in response to the mechanical strain results in enhanced bone formation [70]. The ability of exercise to protect bone during weight loss is supported by a systematic review which found that hip and lumbar spine BMD decreased significantly during CR interventions, but no significant effect on BMD occurred when exercise was implemented during CR [45]. These findings are further supported by the effects on bone turnover markers. A study was conducted observing older obese adults and the effects of CR with and without exercise on bone turnover. During the 1 year intervention, increases in serum c-terminal telopeptide (31%) and

osteocalcin (24%) concentrations occurred during CR, but were maintained during CR with exercise. Decreases in leptin and estradiol, which promote bone formation, also occurred during CR without exercise (Leptin: -25%; Estradiol: -15%) and CR with exercise (Leptin: -38%; Estradiol: -13%) [71]. Although exercise did not prevent the reduction in leptin and estradiol, it seems to reduce bone turnover and prevent the reduction in BMD which occurs during CR, possibly due to the mechanical stress that is placed on the bone during exercise.

Benefits of a High Protein Intake

Increasing dietary protein intake is another method that has often been employed to attenuate the negative effects of weight loss. The intake of a high protein diet promotes increased levels of circulating amino acids, which activates signaling to promote increased MPS and skeletal muscle growth [4]. A systematic review assessing the effects of caloric restriction and high protein intake in body composition support that a high protein intake (1.07-1.60 g/kg) results in prevented the loss of FFM, greater reductions in body weight, and FM than the low protein groups (0.55-0.88 g/kg) [72]. Overall, there is strong evidence that suggests that a high protein intake is able to further prevent the loss of FFM along with promoting greater weight loss and FM loss during CR though improvements in protein balance and increase MPS.

When coupled with resistance exercise, participants consuming a high protein diet have been able to increase FFM while losing greater amounts of FM during CR. An increase in FFM was observed in a study comparing the effects of a low protein intake (0.72-0.84 g/kg) versus a high protein intake (1.33 g/kg) during 16 weeks of caloric

restriction in overweight and obese adult women. During the intervention, participants in each group performed aerobic exercise daily and resistance exercise 2 days per week. After 16 weeks, all groups lost body weight and fat mass, but the high protein group lost more FM (~3 kg) and gained FFM (0.7 kg). The low protein groups either maintained or lost FFM (-0.2-0.7 kg) during the intervention [23]. Increases in FFM were also found in a 4 week intervention in young overweight men. Participants were split into either a low protein intake (1.2 g/kg) or a high protein intake (2.4 g/kg) and performed a combination of resistance exercise and high intensity interval training 6 days per week. After the 4 week intervention, FFM (1.2 kg) increased in the high protein group and lost a greater amount of FM (-4.8 kg) when compared to the low protein group (FFM: 0.1 kg; FM: -3.5 kg) [26]. From this, we can determine that, when performing a combination of resistance and aerobic exercise and consuming a high protein diet, it is possible to increase FFM during periods of CR.

Although resistance exercise coupled with a high protein intake has been shown to have profound effects on FFM, aerobic exercise combined with a high protein intake has also been able to further prevent the negative effects of weight loss. As discussed previously, aerobic exercise during CR has been shown to maintain FFM without a high protein intake and, similar to resistance exercise, incorporating a high protein intake can have additive effects. In overweight women, the effects of a high protein diet (1.14 g/kg) and CR on body composition were compared to the effects of a low protein diet (0.64 g/kg BW). During the 12 week intervention, participants were encouraged to perform physical activity ≥30 min 3 times per week. Both groups lost similar amounts of weight, but a subcategory of participants with high triglycerides in the high protein group lost

more FM (-6.4 kg) than the low protein group (-3.4 kg) [24]. Similar results were found in another study in overweight and obese men and women who underwent a 6 month CR and maintained their habitual physical activity, but were split into either a high protein group (1.2 g/kg) or a low protein group (0.8 g/kg). Both groups lost similar amounts of FM, but FFM was maintained during the high protein group [25]. The results of the above studies show that a high protein coupled with only aerobic exercise is also able to further protect FFM during prolonged periods of CR.

The effects of a high protein intake on body composition have also been highlighted by the effect on MPS during CR. A high protein intake (1.3 g/kg) after 14 days of CR was found to attenuate the loss of MPS in overweight and obese men and women. Post absorptive MPS decreased in all groups, but postprandial MPS was reduced to a lesser extent in the high protein group. These findings suggest that a high protein intake is able to preserve attenuate the reduction in MPS during CR [22]. Positive effects on MPS were also observed when protein is consumed post-exercise. After 5 days of CR, consuming 15-30 g of protein after a single bout of resistance exercise resulted in elevated rates of MPS above resting values in an energy balance [15]. Therefore, evidence suggests that a high protein intake coupled with exercise will promote favorable changes in body composition during CR.

High Protein can also attenuate Bone Loss during CR

Recent research suggests that a high protein intake during CR may further help to maintain the anabolic sensitivity not only of FFM, but also that of bone. A low protein intake results in increased circulating parathyroid hormone (which promote bone

demineralization) and has also been found to increase intestinal calcium absorption, suggesting that a high protein intake may be able to help prevent bone loss. Increased amino acid content have been found to activate the calcium-sensing receptor (CaSR), resulting in reduced parathyroid hormone secretion and promote osteoblast activity [73]. High protein intake has also been able to impact bone through modulation of hormones such as GH and IGF-1. Protein deficiency was found to impair GH secretion, thus resulting in reduced secretion of IGF-1 and impairing growth in a rat model [28]. The impaired secretion of IGF-1 during a low protein intake also suggests that a high protein may be able to attenuate the reduction in IGF-1 during an energy deficit in humans [27], and is supported by the ability of increased circulating amino acids, primarily branch chain amino acids, to increase insulin and IGF-1 secretion [73].

Unfortunately, mixed results have been found regarding the effect of a high protein diet on IGF-1 during an energy deficit. A protein intake of 0.9 g/kg BW attenuated the reduction in IGF-1 during days 4-8 in young men during an 8-day energy deficit when compared to a protein intake of 0.5 g/kg BW [29, 30]. However in another study, a protein intake of 1.63-1.8 g/kg BW failed to prevent the reduction of IGF-1 in young men and women over the course of 12 days to 1 month of CR when compared to a protein intake of 0.8 g/kg BW [31-33]. Although few studies have explored the effects on insulin, a high protein intake does not seem to be able to prevent the suppression of insulin during an energy deficit in obese individuals [22, 24].

If a high protein diet can rescue IGF-1 secretion during energy deficiency, it may be also attenuate the loss of bone. Improved bone strength was found in young boys consuming a high protein intake when exercising [74]. Few have observed the effect of

high protein intake on bone during an energy deficit, but similar increases in bone turnover markers and decreases in calcium excretion were found in both high and low protein groups during an energy deficit [24, 31]. Overall, one can conclude that mechanisms exist which suggest that a high protein intake may be able to attenuate the loss of bone mass during CR. Further research is necessary to determine if these mechanisms are able to overcome the negative effects on bone.

Conclusion

Considering the side-effects of calorie-restricted weight loss, it is clear that CR can have detrimental effects on various non-adipose tissues, particularly when achieved utilizing CR without exercise. The loss of these tissues can result in health detriments such as impaired physical function and performance, reduced circulating glucose and insulin sensitivity, and increased risk for osteoporosis or fractures. Exercise has been shown to be a potential stimulus to attenuate the loss of FFM, maintain some measures of exercise performance, and attenuate the loss of BMD during CR. A high protein intake, especially when exercising, has been able to further maintain or increase FFM and has been suggested to be able to further maintain BMD during periods of CR. Further research is required to determine if these effects persist in short-term, controlled interventions and clarify mixed results regarding the effects on measures of performance and bone turnover.

CHAPTER 3: APPORACH

Study 1:

Experimental Design

This study includes a secondary analysis on data previously collected during an intervention that has been published previously [52], but the data under investigation has not been previously published. To assess the effects of an energy deficit on performance and to determine whether these effects are attenuated by exercise, a repeated-measures 4-way cross-over design was applied. Participants completed two conditions in an energy deficit and two control conditions in a normal energy intake. Participants performed supervised aerobic exercise during one energy deficit condition (CR+EX) and control condition (CON+EX), and no exercise was performed during the other energy deficit condition (CR-EX) and control condition (CON-EX). The amounts of energy consumed and expended in each condition was quantified using the energy availability (EA) method, which, after deducting energy expended during exercise, is the amount of energy left over for the remaining physiological functions during the day [75]. An energy balance is defined as an EA of 40 kcal/kg FFM and CR is defined as an EA of 15 kcal/kg FFM. Each condition lasted 4 days, and the order of the conditions chosen at random (Figure 3.1). Between each condition, participants completed out washout periods with ad libitum diet and exercise for at least 4 days (following control conditions) or 10 days (following CR conditions).

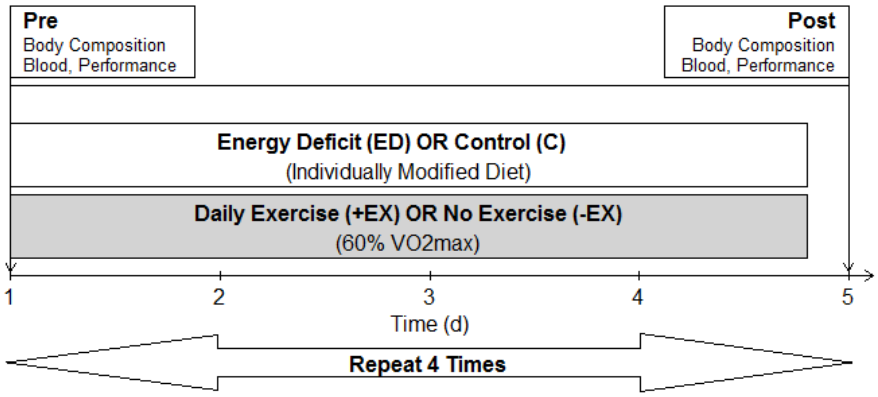

Figure 3.1: Study 1 Procedures Illustration

Participants

Participant were recruited from the campus of the German Sport University Cologne using the following inclusion criteria: male, age: 18-30, ≥3 hours/week of purposeful aerobic exercise, body mass index: 19-25 kg/m^2, ≤15% body fat, and weight stable (±3 kg) during the past 6 months. Participants were excluded if they met any of the following criteria: smoking, past or present diagnosis of a clinical eating disorder, infectious disease within past 4 weeks, cardiovascular disease or orthopedic impairment that would interfere with moderate-to-vigorous exercise, use of medication, and diabetes mellitus. Approval of the study was obtained from the institutional review board of the German Sport University Cologne. Participants provided written informed consent prior to study enrolment.

Preliminary Assessments

Prior to the start of the first condition, participants underwent a nutrition interview and baseline measures of body composition and aerobic performance measures were taken. The nutrition interview was conducted by a trained nutritionist and served to document each participant's diet history, individual dietary habits, and food preferences. Participants reported the frequency, type, and amount of meals, beverages and snacks they consume habitually over the course a day.

Body weight and body composition were assessed using a bioimpedance scale (Tanita BC 418 MA, Tanita, Amsterdam, The Netherlands). Aerobic performance, which was assessed as maximal oxygen uptake (VO_{2max}), as well as exercise intensity and duration for the prescribed exercise bouts were assessed during an incremental exercise on a bicycle ergometer (SRM, Jülich, Germany). Participants began pedaling at a power output of 1.0 W/kg for 5 minutes; resistance was increased to 1.5 W/kg after 5 minutes and to 2.0 W/kg after an additional 5 minutes. Once participants completed 5 minutes at 2 W/kg, resistance was increased by 20 W every 30 seconds until volitional exhaustion, which occurred when ≥ 3 of the following criteria were met: cadence <60 rpm, respiratory exchange ratio ≥1.1, heart rate ≥90% of age-predicted maximum (220 – age), plateau in oxygen uptake despite increase in load, and rating of perceived exertion (RPE) ≥19 (Borg, 1970). Throughout the test inspired oxygen (VO2) and expired carbon dioxide (VCO2) were assessed using a portable spirometric system (Metamax 3B, CORTEX, Leipzig, Germany), which was calibrated with a 3-Liter syringe and room air. Energy expenditure at submaximal exercise intensities was calculated from respiratory data using the Weir equation (Weir, 1949).

Energy Intake and Expenditure Prescriptions

The prescribed EA levels were attained by manipulating dietary energy intake individually such that target energy availabilities of 15 kcal/kg FFM/day (CR conditions) or 40 kcal/kg FFM/day (CON conditions) were attained after accounting for exercise energy expenditure [61]. Exercise energy expenditure (ExEE) was set at 15 kcal/kg FFM/day during both exercising conditions and was attained by having participants cycle at 60% of their VO_{2max} until the prescribed ExEE was attained. We chose to reduce EA to 15 kcal/kg FFM based on previous experiments, demonstrating that endocrine effects were more pronounced when EA was reduced to 10-20 kcal/kg FFM/day when compared to 30 kcal/kg FFM/day [76-80]. In our control condition, EA was 40 kcal/kg FFM/day, which was lower than the 45 kcal/kg FFM/day that had been operationally defined as balanced EA by Loucks and colleagues [76-80]. We opted to maintain EA during control conditions at 40 kcal/kg FFM/day, which is supported by field data demonstrating that the habitual EA of exercising men is closer to 40 than 45 kcal/kg FFM/day [81]. During non-exercising conditions, ExEE was 0 kcal/kg FFM/day. ExEE was adjusted for habitual waking energy expenditure, which represents the energy expenditure that would have occurred in absence of the prescribed exercise bout. This was achieved by multiplying the duration of the exercise bout with the participants habitual waking energy expenditure, which was calculated as resting energy expenditure (REE) multiplied by the a physical activity level (PAL) of 1.7. A PAL of 1.7 was selected to characterize a lifestyle with a moderate amount of activity [82]. Resting energy expenditure was derived from FFM [83].

Diet Prescriptions

A thorough meal plan, describing the amount and kind of foods that were allowed, was given to participants during each condition, and diet tendencies and food preferences, as reported during the initial nutrition interview were used to create the meal plans individually for each participant. Energy intake was distributed across 4-6 meals and snacks throughout the day and the amount of meals chosen initially by the participant was maintained the same across all conditions. Meal sizes were adjusted so that daily EI matched EI prescriptions for each condition. Efforts were made to maintain a macronutrient composition within the recommended ranges of the German Nutrition Society (50-55% carbohydrates, 30-35% fat, 10-15% protein (DGE, 2012)). The type and amount of foods chosen were explained to participants when they received the meal plan. Non-caloric beverages and non-caloric sweeteners were allowed during the study. Food scales were provided to weigh all food consumed and left over food. Participants were encouraged to report any deviation from the prescribed meal plan to the study personnel. On a daily basis, dietary EI was analyzed using EBIS software (version 7.0, University of Hohenheim, Stuttgart, Germany, 2005). If the actual EI deviated from prescribed daily EI by more than 50 kcals on any given day, the meal plans for subsequent days were adjusted to reach the average goal EI over the whole condition.

Exercise Expenditure

During both exercising conditions, participants performed supervised daily exercise on a bicycle ergometer (SRM, Jülich, Germany). Intensity was set at 60%

VO_{2max} and participants exercised until they had expended a total of 15 kcal/kg FFM/day. Participants were not allowed to perform any additional exercise or intense physical activity. The SenseWear Pro3 armband (Bodymedia, Pittsburgh, USA) was utilized to monitor abstention from exercise and intense physical activity.

Measurements and Assessments

Before and after the completion of each 4-day condition, all tests were conducted in the same order. After an overnight fast of at least 12 h and refraining from exercise for at least 18 h, participants arrived at the lab, and provided an initial urine sample. If the sample indicated dehydration, defined by a specific gravity >1.020 g/mL [84], participants were asked to consume 500 mL of tap water and provided another urine sample before proceeding through the remaining assessments. Body weight and composition were measured as described for the preliminary assessments. Participants were asked to fill out a survey consisting of 32 adjectives to assess perceived physical state as well as psychological strain and motivational state using a handheld computer which has been described previously [85]. Participants were asked to indicate how well each adjective matched their current mental or physical state on a range of 0 (none) to 5 (exactly). Time allowed for each question was limited to 5 seconds to discourage rational deliberation. All of the adjectives and instructions were provided in German and have been translated to English to be used in this manuscript. The 32 adjectives were broken down into the following sub-dimensions: physical energy, which includes adjectives associated with lethargy and tiredness (“drained”, “weak”, “weary”, “shiftless”), physical fitness (“well-trained”, “vigorous”, “fit”, “strong”), physical health (“healthy”, “groggy”,

"battered", "sick"), physical flexibility ("agile", "flexible", "immobile", "stiff"), positive mood ("positive mood", "cheerful"), calmness ("relaxed", "calm"), recovery ("recuperated", "well-rested"), relaxation ("feeble", "drowsy"), willingness to seek contact ("open for contact", "communicative"), social acceptance ("accepted", "popular"), readiness to strain ("energetic", "powerful") and self-confidence ("experienced", "self-confident").

Maximal and submaximal measures of aerobic performance (VO_{2max}, fat oxidation, heart rate, and ratings of perceived exertion (RPE)) were assessed during an incremental exercise test, which was identical to the incremental test performed during the initial assessments. Submaximal measures were collected during steady state conditions during the initial stages of the test (1.0, 1.5, and 2.0 W/kg), which each lasted 5 minutes.

Anaerobic performance of the lower body was assessed using a 30-second Wingate test on a bicycle ergometer (SRM, Jülich, Germany). Following completion of the incremental exercise test, participants cycled at 1 W/kg for 10 minutes to allow for active recovery, after which the participant was instructed to pedal as fast as possible for 30 seconds against the internal resistance of the ergometer, which was achieved by setting maximal revolutions per minute (RPM) to 120 with an unlimited wattage. Strong verbal encouragement was given and participants were required to stay seated during the entire test. Peak power, mean power, and fatigue index were determined, and fatigue index was calculated as peak power minus minimum power divided by peak power [86].

Isometric strength was assessed on a bench press machine, leg extension machine and leg curl machine (gym80; Gelsenkirchen, Germany). Following at least 10 minutes of

passive recovery, participants performed three attempts of each exercise in a random order. The following settings were adjusted for each of the following tests: bench press (60° abduction in shoulder, 30° horizontal adduction (shoulder)), leg extension (inner knee angle 120°, maximal dorsiflexion), and leg curl (inner knee angle 150°). Following a countdown ("3, 2, 1"), participants were instructed to attempt to produce as much force as possible against the resistance and were verbally encouraged by the study personnel. Strength measures were recorded using 100 Hz frequency and were analyzed using DigiMax Iso Test software (Version 2.0, DigiMax Messtechnik, Hamm, Germany) to determined maximal force and rate of force development.

Statistical Analysis

Statistical analyses was performed with R (version 3.3.2, The R Foundation for Statistical Computing). If not stated otherwise, data was reported as mean and upper and lower 95% confidence interval (95% CI). Linear mixed model analysis was used to identify differences in study outcomes and included fixed effect terms for changes due to CR ('CR'), changes due to exercise ('EX'), and the interaction between CR and exercise ('CRxEX'). To account for repeated measures, the participant identifier was included as a random effect. When time or interaction effects occur ($p<0.1$), post-hoc analyses was conducted using paired Wilcoxon rank sum tests. Significance was set at $p<0.05$, and was adjusted for multiple testing.

Study 2:

Experimental Design

A repeated-measures 3-way cross-over design was be applied in the second study. Participants completed two conditions in a low EA (15 kcal/kg FFM/day) and one control condition in a normal EA (40 kcal/kg FFM/day). Participants also consumed a high protein intake (1.7 g·kg^{-1} BW) during one low EA condition (CR+P) and the normal EA condition (CON), and a normal protein intake (0.8 g·kg^{-1} BW) during the other low EA condition (CR-P). Each condition lasted 5 days, and the order of the conditions was block randomized (Figure 3.2). Between each condition, participants completed washout periods with ad libitum diet and exercise for at least 14 days to allow protein balance to return to baseline [87].

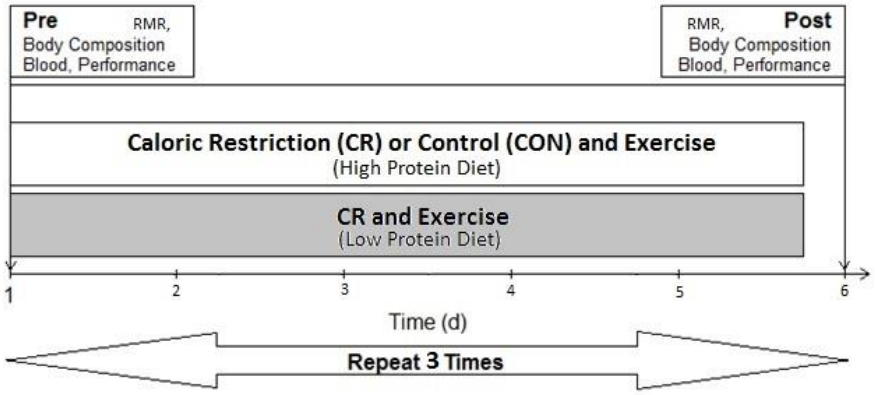

Figure 3.2: Study 2 Procedures Illustration

Participants

Participant were recruited from the campus of the University of Nebraska-Lincoln using the following inclusion criteria: male, age: 18-30, ≥4 hours/week of purposeful aerobic exercise, body mass index: 19-25 kg/m2, ≤15% body fat, and weight stable (±2.5

kg) during the past 6 months. Participants were excluded if they meet any of the following criteria: smoking, past or present diagnosis of a clinical eating disorder, infectious disease within past 4 weeks, cardiovascular disease or orthopedic impairment that would interfere with moderate-to-vigorous exercise, use of medication, and diabetes mellitus. Approval of the study was obtained from the institutional review board of the University of Nebraska Lincoln. Participants were provided written informed consent prior to study enrolment.

Preliminary Assessments

Body weight and body composition were assessed using a bioimpedance analyzer (Bodystat Quadscan 4000). Aerobic performance, assessed as maximal oxygen uptake (VO_{2max}), as well as exercise intensity and duration for the prescribed exercise bouts were assessed during an incremental exercise on a bicycle ergometer (Monark LC6; Vansbro, Sweden). Participants began pedaling at a power output of 60 W for 3 minutes; resistance was be increased by 35 W every 3 minutes until volitional exhaustion, which occurred when ≥ 3 of the following criteria were met: cadence <60 rpm, respiratory exchange ratio ≥1.1, heart rate ≥90% of age-predicted maximum (220 – age), plateau in oxygen uptake despite increase in load, and rating of perceived exertion (RPE) ≥19 [88]. Throughout the test inspired oxygen (VO_2) and expired carbon dioxide (VCO_2) were assessed using a portable spirometric system (COSMED Quark CPET Metabolic Testing System; Rome, Italy), which was calibrated with a calibrated 3-Liter syringe using room air. Energy expenditure at submaximal exercise intensities was calculated from respiratory data using the Weir equation [89]. Participants also filled out questionnaires to assess eating

disorder tendencies, health history and nutrition, calcium intake, physical activity impacting bone density, and contraindications to exercise testing.

Energy Intake and Expenditure Prescriptions

The prescribed EA levels were attained by manipulating dietary EI individually such that target energy availabilities of 15 kcal/kg FFM/day (CR conditions) or 40 kcal/kg FFM/day (CON condition) were attained after accounting for exercise energy expenditure [61]. Exercise energy expenditure was set at 15 kcal/kg FFM/day during both exercising conditions, which was attained by having participants cycle at 60% of their VO_{2max} until the prescribed exercise energy expenditure was attained. As done in study one, exercise expenditure was adjusted for habitual waking energy expenditure.

Diet prescriptions

Dietary energy intake prescriptions were single blind and controlled individually utilizing a combination of two clinical products (Ensure Original and Ensure High Protein, both Therapeutic Nutrition, Abbot) and maltodextrin (Tate & Lyle) such that the target energy intake of 30 kcal/kg FFM/day (CR-P and CR+P) or 55 kcal/kg FFM/day (CON) is reached. Participants were provided clear plastic bottles each day with the prescribed amount of the clinical products and were asked to consume all of it during the day distributed across 3-5 meals and snacks. Maltodextrin was provided dissolved in water as part of the hydration during the prescribed exercise bouts. Participants were supplemented with calcium and vitamin D at least 2 weeks prior to study start and throughout the duration of the conditions and washout periods to maintain calcium and

Vitamin D intake at a constant level. Participants were provided with intake logs to track their consumption. In addition to the provided products, participants were only be allowed to consume water and non-caloric beverages.

Daily Procedures and Exercise Expenditure

Prior to each condition, participants were provided with equipment for urine collection (measuring cup, collection tubes) and three urine samples per day will be collected in days 1-5 (first void of the day, post-exercise, and in the evening). Urine samples were analyzed for ketones (a qualitative marker of caloric deficiency) using urine ketone sticks (KETOSTIX Reagent Strips for Urinalysis, Bayer) to assess compliance. During each condition on days 1-5, participants conducted daily supervised exercise on a bicycle ergometer (Monark LC6) to expend the prescribed amount of calories as done in study 1. Before the exercise, participants filled out questionnaires assessing mood state and satiety. After exercise, a post-exercise urine sample was taken while the participant is in the laboratory. Additional exercise and intense physical activity was prohibited. Compliance was monitored using an activity monitor (Actigraph version 6.13.3) during each condition.

Preliminary and Post-condition Measurements and Assessments

Before and after the completion of each 5-day condition, all tests were conducted in the same order. After an overnight fast of at least 12 hours, participants arrived at the lab, and were provided an initial urine sample. Body weight and composition were

measured as described for the preliminary assessments. Participants were then asked to fill out the following questionnaires assessing mood state, eating behavior, and satiety.

A fasting blood sample was taken from the forearm vein while lying in a supine position. Fasting samples were used to measure insulin, IGF-1, IGF-binding protein 3, and testosterone. For these analyses, commercially available enzyme-linked immunoassays from R&D Systems, Inc., Minneapolis, MN, were used.

After blood collection, resting metabolic rate (RMR) was measured. Participants were asked to lie supine at rest for 30 minutes in order to achieve a steady state prior to measurement. Oxygen consumption and carbon dioxide production were then be measured with a ventilated hood system (COSMED Quark CPET Metabolic Testing System; Rome, Italy) for 30-45 minutes. RMR was calculated from oxygen uptake and carbon dioxide production using the Weir equation (Weir, 1949).

Maximal and submaximal measures of aerobic performance (VO_{2max}, fat oxidation, heart rate, and ratings perceived exertion (RPE)) were assessed during an incremental exercise test, which will be identical to the incremental test performed during the initial assessments.

Statistical Analysis

Statistical analyses were performed with R (version 3.3.2, The R Foundation for Statistical Computing). If not stated otherwise, data was reported as mean and upper and lower 95% confidence interval (95% CI). With respect to the potential small sample size, only non-parametric tests were applied. Linear mixed model analysis were used to identify differences in study outcomes and included fixed effect terms for changes from

pre to post measurements (‘time’) and interaction between time and CR (‘timexCR’), between time and protein (‘timexP’), and between time, CR, and protein (‘timexCRxP’). To account for repeated measures, the participant identifier was included as a random effect. When time or interaction effects occur ($p<0.1$), post-hoc analyses were conducted using paired Wilcoxon rank sum tests. Significance was set at $p<0.05$, and was adjusted for multiple testing. Due to the small sample size and the exploratory nature of the study, $p<0.1$ was used to detect trends.

CHAPTER 4: RESULTS

Study 1

Demographics

All six participants completed each of the 4 conditions. The participants were 25.2 ± 1.0 (mean ± standard error of the mean) years of age, weighed 79.7 ± 3.1 kg, had a body fat percentage of 9.6 ± 1.5%, and a VO_{2peak} of 49.3 ± 2.4 ml/kg/min.

Anthropometrics

Participants lost significant amounts of weight ($p < 0.01$) during both CR conditions (CR+EX: -1.8 kg [-2.6; -1.0]; CR-EX: -2.4 kg [-3.0; -1.9]); whereas, no weight was lost during either control condition (Figure 4.1). Fat free mass tended to decrease during CR-EX ($p = 0.07$), but not during the CR+EX ($p = 0.13$).

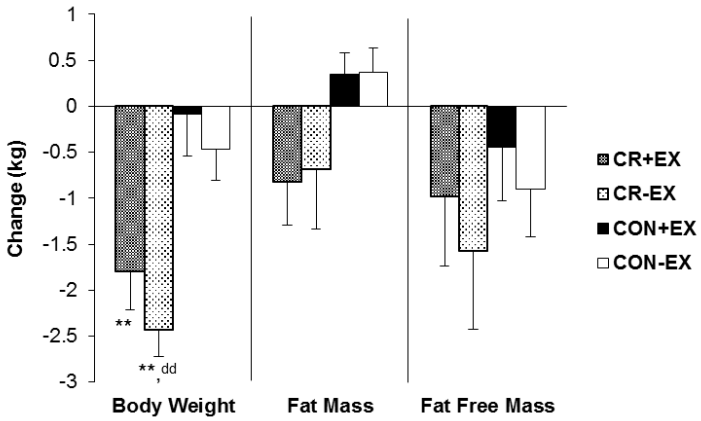

*Figure 4.1: Changes in body weight, fat mass, and fat free mass over the course of each 4-day intervention, consisting of caloric restriction (CR; 15 kcal/kg fat free mass (FFM)) with (+EX) or without (-EX) and normal energy availability (CON; kcal/kg FFM) with or without exercise. Bars represent the group mean, and error bars represent the standard error of the mean.**indicates a change that is significantly different from zero ($p < 0.01$). dd indicates a change that is significantly different from the change in CON-EX ($p < 0.01$).*

Strength

Leg curl and leg extension maximal force, when corrected for changes in body weight, did not change significantly during any condition (Figure 4.2). There was a trend indicating an increase in leg curl rate of force development during CR-EX ($p = 0.07$) and there was also a trend indicating an increase in leg extension rate of force development during both CR conditions (CR+EX: $p = 0.08$; CR-EX: $p = 0.07$). Bench press maximal force, when corrected for changes in body weight, decreased by 6.4 % [-12.2; -0.6] during CR+EX ($p = 0.04$), but did not change significantly during CR-EX ($p = 0.36$). Bench press rate of force development increased during CR+EX ($p = 0.05$), but did not change during CR-EX ($p = 0.20$).

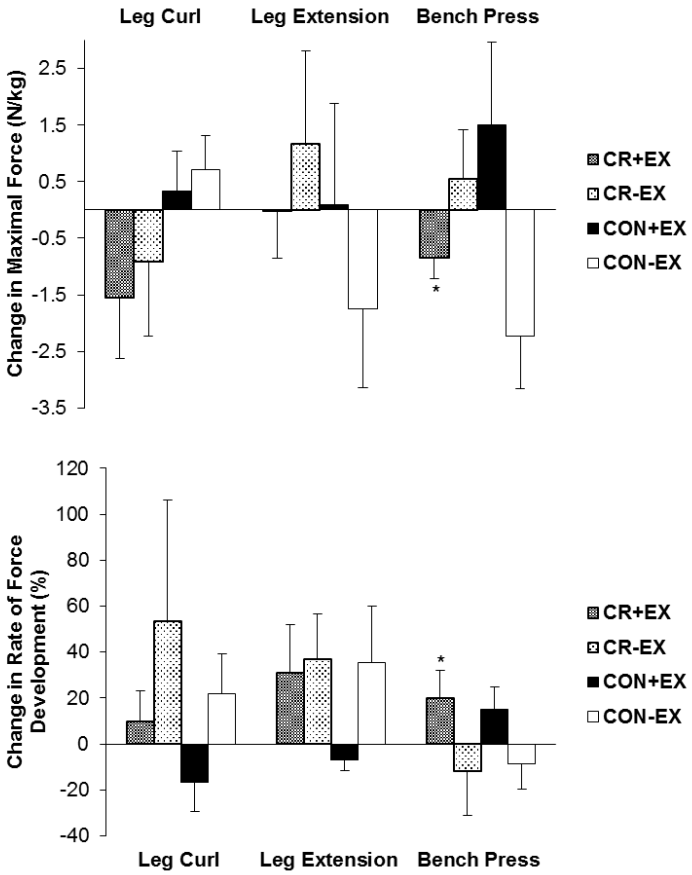

*Figure 4.2: Changes in maximal force and rate of force development during leg curl, leg extension, and bench press over the course of each 4-day intervention, consisting of caloric restriction (CR; 15 kcal/kg fat free mass (FFM)) with (+EX) or without (-EX) and normal energy availability (CON; kcal/kg FFM) with or without exercise. Bars represent the group mean, and error bars represent the standard error of the mean. * indicates a change that is significantly different from zero ($p < 0.05$).*

Aerobic and Anaerobic Performance

VO_{2peak}, when normalized for changes in body weight, increased by 6.2 % [2.8; 9.5] during CR+EX ($p < 0.01$), and there was also a trend indicating an increase in VO_{2peak} during CON+EX ($p = 0.06$; Table 1). No significant changes in VO_{2peak} were found during CR-EX ($p = 0.26$) and CON-EX ($p = 0.11$). When normalized for body weight, power output at VO_{2peak} increased by 3.7 % [1.4; 7.0] during CR-EX ($p < 0.01$), but did not change significantly during all other conditions. Maximal power generated during the Wingate test, when normalized for body weight, did not change significantly during any condition (Table 4.1). Mean Wingate power tended to decrease during CR+EX ($p = 0.07$) and to increase during CR-EX ($p = 0.06$). The fatigue index tended to increase during CR+EX ($p = 0.07$). No changes in fatigue index were found during other conditions (Table 4.3).

Table 4.1. Changes in measures of maximal aerobic fitness during each condition (mean; 95% confidence interval)

	VO_{2peak}		Power at VO_{2peak}	
	L/min	**mL/kg/min**	**W**	**W/kg**
CR+EX				
Day 1	4.1 [3.7; 4.5]	51.0 [48.1; 53.9]	388 [344; 431]	4.8 [4.3; 5.4]
Day 5	4.3 [3.8; 4.7]	54.2 [51.0; 57.3]	383 [342; 424]	4.9 [4.4; 5.4]
Change	**0.1 [0.0; 0.3]***	**3.1 [1.5; 4.8]****	-5 [-12; 2]	0.1 [0.0; 0.1]
CR-EX				
Day 1	3.8 [3.6; 4.0]	48.0 [44.3; 51.6]	391 [353; 429]	4.9 [4.5; 5.3]
Day 5	3.8 [3.4; 4.1]	48.8 [44.9; 52.7]	393 [356; 429]	5.1 [4.6; 5.6]
Change	0.0 [-0.4; 0.3]	0.8 [-3.3; 5.0]	2 [-8; 11]	**0.2 [0.1; 0.3]*[a]**
CON+EX				
Day 1	3.7 [3.3; 4.1]	47.0 [39.8; 54.3]	391 [348; 434]	5.0 [4.4; 5.5]
Day 5	3.9 [3.7; 4.2]	48.8 [44.6; 55.4]	394 [358; 430]	5.0 [4.5; 5.5
Change	0.2 [-0.1; 0.5]	0.8 [-1.1; 7.0]	3 [-12; 18]	0.0 [-0.1; 0.2]
CON-EX				
Day 1	4.1 [3.7; 4.4]	51.2 [47.6; 54.8]	413 [387; 439]	5.2 [4.7; 5.8]
Day 5	4.2 [3.8; 4.7]	53.7 [49.2; 58.1]	408 [387; 429]	5.2 [4.7; 5.7]
Change	**0.2 [0.0; 0.3]***	2.5 [0.4; 4.5]	-5 [-13; 3]	0.0 [-0.1; 0.1]

* denotes difference from zero ($p < 0.05$) and ** ($p < 0.01$)

[a] denotes difference from CR+EX ($p < 0.05$)

[d] denotes difference from CON-EX ($p < 0.05$)

Table 1. Changes in submaximal aerobic fitness during each condition (mean ; 95% confidence interval)

	Heart Rate				RPE		
	1 W/kg	**1.5 W/kg**	**2 W/kg**	**Maximal**	**1 W/kg**	**1.5 W/kg**	**2 W/kg**
CR+EX							
Day 1	102 [95; 109]	117 [112; 121]	136 [131; 140]	183 [172; 193]	9 [7; 10]	10 [9; 12]	13 [12; 13]
Day 5	102 [92; 112]	122 [115; 130]	140 [134; 145]	182 [175; 190]	9 [8; 11]	11 [9; 13]	13 [11; 15]
Change	0 [-10; 10]	6 [-2; 13]	4 [-3; 11]	0 [-3; 3]	1 [0; 1]	1 [0; 2]	1 [-1; 2]
CR-EX							
Day 1	98 [90; 107]	114 [105; 124]	132 [123; 141]	183 [172; 193]	9 [7; 11]	11 [10; 13]	13 [12; 14]
Day 5	105 [97; 115]	121 [113; 130]	142 [132; 151]	187 [177; 197]	10 [8; 11]	12 [10; 13]	14 [13; 15]
Change	**7 [3; 11] ****	**7 [4; 10] ****	**10 [5; 14] ****	4 [0; 8]	1 [-1; 2]	0 [0; 1]	**1 [0; 2]***
CON+EX							
Day 1	107 [95; 119]	121 [111; 131]	138 [130; 147]	187 [178; 196]	8 [6; 9]	10 [9; 11]	12 [11; 14]
Day 5	102 [93; 112]	119 [108; 129]	134 [126; 142]	185 [177; 193]	9 [8; 10]	11 [10; 12]	12 [11; 13]
Change	-5 [-13; 3]	-2 [-9; 5]	-4 [-15; 7]	-2 [-6; 1]	**1 [0; 3][d]**	1 [0; 1]	0 [-1; 1]
CON-EX							
Day 1	102 [96; 108]	118 [111; 125]	134 [128; 139]	184 [178; 189]	11 [9; 12]	11 [10; 12]	13 [12; 14]
Day 5	103 [97; 110]	120 [113; 128]	137 [129; 145]	185 [178; 192]	8 [7; 9]	10 [9; 11]	13 [12; 14]
Change	2 [-4; 7]	3 [-5; 10]	3 [-2; 8]	1 [-2; 4]	**-3 [-5; 0]***	**-1 [-2; 0]***	0 [-1; 1]

* denotes difference from zero ($p < 0.05$) and ** ($p < 0.01$)

[a] denote difference in change from CR+EX ($p < 0.05$)

[d] denotes difference in change from C-EX ($p < 0.05$)

Table 4.3. Change in measures of anaerobic fitness during each condition (mean; 95% confidence interval)

	Maximal power (N/kg)	Mean power (N/kg)	Fatigue index (%)
CR+EX			
Day 1	13.4 [11.5; 15.5]	8.8 [7.8; 10.0]	34.2 [26.0; 42.5]
Day 5	13.2 [10.8; 15.7]	8.5 [7.3; 9.9]	40.5 [38.7; 42.5]
Change (%)	-2.3 [-7.8; 3.3]	**-4.4 [-9.3; 0.5]**[a]	29.8 [-6.8; 66.3]
CR-EX			
Day 1	13.7 [12.5; 15.0]	7.9 [6.5; 9.4]	39.9 [35.8; 44.0]
Day 5	14.4 [13.0; 15.9]	9.0 [8.0; 9.9]	39.2 [34.5; 43.8]
Change (%)	5.4 [-0.9; 11.7]	15.8 [-2.3; 33.8]	-1.5 [-11.6; 8.7]
CON+EX			
Day 1	13.8 [11.9; 15.7]	9.2 [8.3; 10.3]	38.0 [35.7; 40.3]
Day 5	13.9 [11.8; 16.1]	9.7 [9.0; 10.3]	37.5 [26.2; 48.8]
Change (%)	1.2 [-5.2; 7.7]	5.5 [-3.3; 14.3]	-0.9 [-30.3; 28.5]
CON-EX			
Day 1	14.2 [12.2; 16.2]	9.5 [8.6; 10.5]	41.4 [36.6; 46.2]
Day 5	14.0 [12.1; 15.9]	9.6 [8.6; 10.8]	44.1 [38.1; 50.0]
Change (%)	1.2 [-5.0; 3.0]	1.4 [-6.6; 9.5]	7.9 [-9.5; 25.4]

[a] denotes difference from CR-EX ($p < 0.05$).

Submaximal Measures

There was a fixed effect of CR suggesting that resting heart rate increased during CR ($p = 0.04$), although post-hoc analyses failed to confirm significant increases in resting heart rate for CR-EX ($p = 0.71$) and CR+EX ($p = 0.34$). Submaximal heart rate increased during CR-EX at all intensities ranging from 1-2 W/kg ($p < 0.01$); whereas, no significant changes in heart rate were observed during the other conditions. RPE at a power output of 2 W/kg increased by 1 units [0; 2] during CR-EX ($p = 0.05$); No changes in submaximal RPE were found during CR+EX and both control conditions (Table 4.2). Rates of fat oxidation during submaximal exercise increased during both CR conditions at intensities of 1 W/kg (CR+EX: 0.3 g/min [0.1; 0.4], CR-EX: 0.2 g/min [0.1; 0.4]; $p < 0.01$) and 1.5 W/kg (1 W/kg: 1.5 W/kg: CR+EX: 0.4 g/min [0.2; 0.5], CR-EX: 0.3 ± 0.0 g/min [0.2; 0.4]; $p < 0.01$) (Figure 4.3). The increase in rates of fat oxidation at 2 W/kg was only significant during CR-EX (0.3 g/min [0.1; 0.5]; $p = 0.02$). Submaximal fat oxidation did not change significantly during both control conditions.

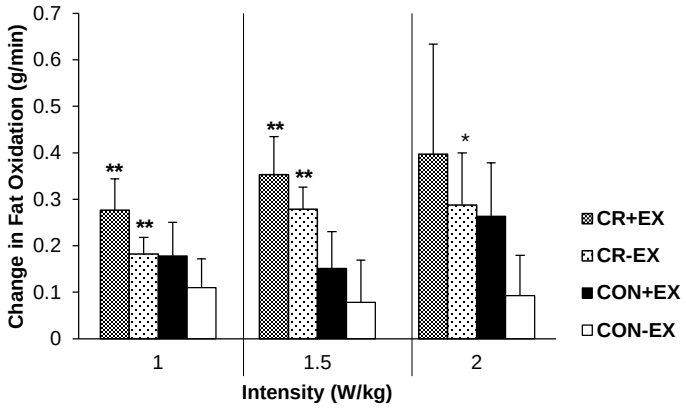

*Figure 4.3: Changes in fat oxidation rates over the course of each 4-day intervention at submaximal intensities of 1, 1.5 and 2 W/kg, consisting of caloric restriction (CR; 15 kcal/kg FFM) with (+EX) or without (-EX) and normal energy availability (CON; 40 kcal/kg FFM) with or without exercise. Bars represent the group mean, and error bars represent the standard error of the mean. * indicates a change that is significantly different from zero ($p < 0.05$); **indicates a change that is significantly different from zero ($p < 0.01$).*

Psychological Indices of Well-being

Several indices of well-being, including positive mood ($p = 0.04$), self-confidence ($p = 0.02$), sense of fitness ($p = 0.08$), physical energy ($p = 0.06$), recovery ($p = 0.07$), relaxation ($p=0.07$), and seeking for contact ($p < 0.01$) decreased during CR-EX (Table 4.4); whereas, these parameters were not significantly altered during CR+EX.

Table 4.4: Changes in mood assessments over the course of each 4-day intervention. The scale ranged from 0 (not at all) to 5 (exactly) (mean; 95% confidence interval)

	CR+EX	CR-EX	CON+EX	CON-EX
Fitness	-0.4 [-1.4; 0.5]	-1.0 [-2.2; 0.2]	-0.5 [-1.3; 0.3]	0.0 [-0.2; 0.2]
Physical Energy	-0.4 [-1.3; 0.5]	-1.2 [-2.5; 0.1]	-0.7 [-1.6; 0.3]	0.3 [-0.2; 0.7]
Flexibility	-0.1 [-0.8; 0.6]	-0.4 [-1.6; 0.9]	-0.4 [-1.2; 0.3]	-0.3 [-0.5; 0.0]
Health	-0.0 [-0.7; 0.6]	-0.2 [-1.4; 1.0]	-0.3 [-1.2; 0.6]	0.3 [0.0; 0.6]
Positive Mood	0.0 [-0.7; 0.7]	**-0.6 [-1.1; -0.1]***	0.1 [-0.3; 0.5]	0.2 [-0.6; 1.0]
Recovery	-1.1 [-2.5; 0.3]	-1.4 [-3.0; 0.2]	-0.3 [-1.7; 1.1]	0.0 [-0.3; 0.3]
Readiness	-0.8 [-2.1; 0.6]	-1.1 [-2.6; 0.5]	-0.1 [-0.7; 0.5]	-0.2 [-0.5; 0.2]
Self-confidence	-0.1 [-0.5; 0.3]	**-0.4 [-0.7; -0.1]***	0.3 [-0.6; 1.2]	0.3 [-0.2; 0.7]
Relaxation	-0.5 [-1.8; 0.8]	-1.0 [-2.1; 0.1]	0.2 [-0.5; 0.9]	**0.4 [0.1; 0.7]***
Seeking for Contact	0.4 [-0.2; 1.1]	**-0.8 [-1.1; -0.4]****	0.1 [-0.4; 0.6]	0.4 [-0.1; 0.9]
Social Acceptance	-0.2 [-0.5; 0.2]	-0.3 [-0.9; 0.4]	-0.2 [-0.6; 0.2]	0.0 [-0.6; 0.6]
Calmness	0.0 [-0.5; 0.5]	-0.3 [-0.7; 0.2]	**-0.6 [-0.8; -0.4]****	0.3 [-0.3; 0.9]

* denotes difference from Day 1 ($p < 0.05$) and ** ($p < 0.01$)

Study 2

Study Progress and Demographics

Thirteen participants reenrolled in the study and signed informed consent documents. Two participants completed each of the 3 conditions. Three participants completed just 1 condition before deciding not to continue the study. One participant withdrew from the study due a minor adverse event (figure 4.4). The participants who completed at least 1 condition were 21.2 ± 1.3 years of age, weighed 81.9 ± 3.5 kg, had a body fat percentage of 14.5 ± 1.7%, and a VO_{2peak} of 43.6 ± 2.7 ml/kg/min.

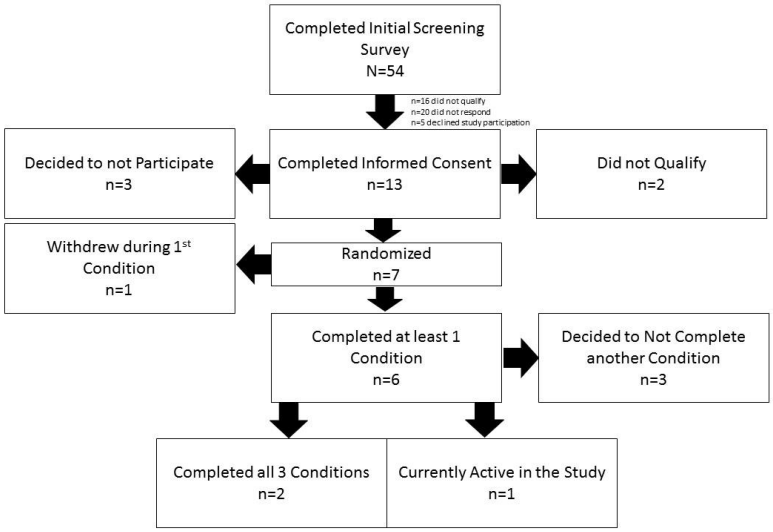

Figure 4.4: Participant study progress.

Anthropometrics

Body weight decreased by 2.5 kg [-3.0; -2.1] during CR+LP ($p < 0.01$) and 3.5 kg [-4.9; -2.0] during CR+HP ($p < 0.01$). No change in body weight was found during CON. Changes in FFM and FM failed to reach significance (figure 4.5). Although no significant changes in FFM and FFM were found, only 22.2% [-217.3; 261.8] of the weight was lost was from FFM during CR+HP ($p = 0.06$) unlike during CR+LP where 63.9% [3.3; 124.5] was lost from FFM ($p < 0.01$).

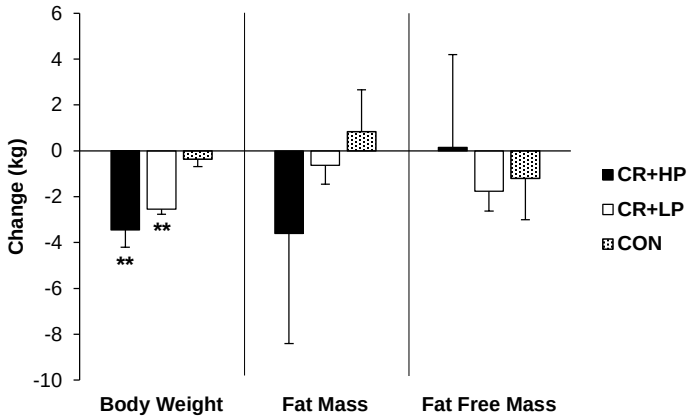

*Figure 4.5: Changes in body weight, fat mass, and fat free mass over the course of each 5-day intervention, consisting of caloric restriction (CR; 15 kcal/kg fat free mass (FFM)) with (+HP) or without (+LP) a high protein intake and normal energy availability (CON; kcal/kg FFM). Bars represent the group mean, and error bars represent the standard error of the mean. **indicates a change that is significantly different from zero ($p < 0.01$).*

Aerobic Performance and Metabolism

VO_{2peak} tended to increase by 6.8 mL/kg/min [2.7; 10.8] during CR+HP ($p = 0.10$), but did not change significantly during all other conditions. Peak power increased by 0.55 W/kg BW [-0.18; 1.28] during CR+HP ($p = 0.04$), but did not change significantly during CR+LP (Table 4.5). Maximal fat oxidation increased by 0.34 g/min [0.32; 0.36] during CR+HP ($p = 0.02$) and tended to increase by 0.15 g/min [-0.03; 0.33] during CR+LP ($p = 0.09$). No changes in maximal fat oxidation occurred during CON. No changes in intensity at maximal fat oxidation seemed to occur during all conditions (figure 4.6).

Table 4.5. Changes in Maximal Aerobic Fitness During each Condition (mean; 95% confidence interval)

	VO_{2peak}		Peak Power	
	L/min	**mL/kg/min**	**Watts**	**W/kg**
CR+HP				
Day 1	3.7 [3.5; 4.0]	43.0 [39.9; 46.1]	270 [201; 339]	3.1 [2.3; 3.9]
Day 6	4.1 [3.6; 4.7]	49.8 [42.6; 56.9]	305 [0; 0]	3.7 [3.6; 3.7]
Change	**0.4 [0.2; 0.7]**	**6.8 [2.7; 10.8]**	35 [-34; 104]	0.5 [-0.2; 1.3]
CR+LP				
Day 1	3.6 [3.2; 3.9]	43.2 [35.9; 50.5]	291 [264; 318]	3.5 [2.9; 4.2]
Day 6	3.4 [3.1; 3.7]	42.4 [34.4; 50.4]	277 [251; 303]	3.5 [2.8; 4.2]
Change	-0.2 [-0.1; 0.2]	-0.8 [-6.1; 4.6]	-14 [-31; 3]	-0.1 [-0.3; 0.2]
CON				
Day 1	3.5 [3.1; 3.9]	41.3 [35.8; 46.9]	293 [270; 316]	3.4 [3.1; 3.7]
Day 6	3.4 [3.0; 3.8]	40.4 [35.0; 45.7]	270 [230; 310]	3.2 [2.7; 3.7]
Change	-0.1 [-0.4; 0.3]	**-1.0 [-5.3; 3.4]**	**-23 [-46; 0]**	-0.3 [-0.6; 0.0]

* denotes difference from zero ($p < 0.05$) and ** ($p < 0.01$)

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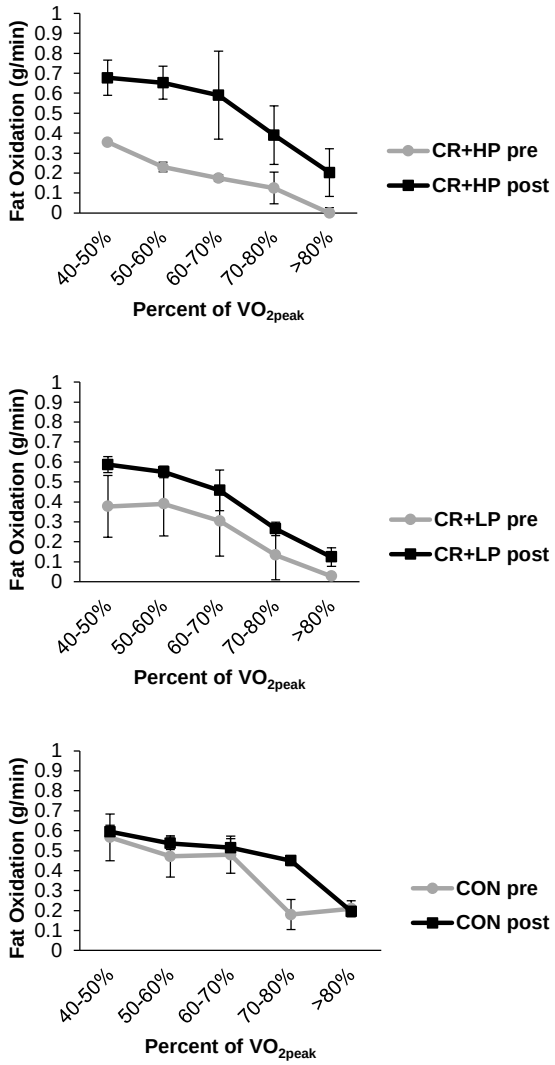

Figure 4.6: Changes fat oxidation over the course of each 5-day intervention, consisting of caloric restriction (CR; 15 kcal/kg fat free mass (FFM)) with (+HP) or without (+LP) a high protein intake and normal energy availability (CON; kcal/kg FFM). Bars represent the group mean, and error bars represent the standard error of the mean.

Submaximal Performance

Average heart rate during prescribed exercise decreased by 5.5 bpm/day [-5.5; -5.4] during CR+HP (p = 0.01), decreased by 2.2 bpm/day [-4.7; 0.3] during CR+LP (p = 0.07) and decreased by 3.6 bpm/day [-5.1; -2.0] during CON (p = 0.03). RPE during prescribed exercise decreased by 0.7 units/day [-0.8; -0.5] during CR+HP (p = 0.01) and decreased by 0.4 units/day [-0.9; 0.13] (p = 0.06) during CON, but did not change significantly during CR+LP (figure 4.7).

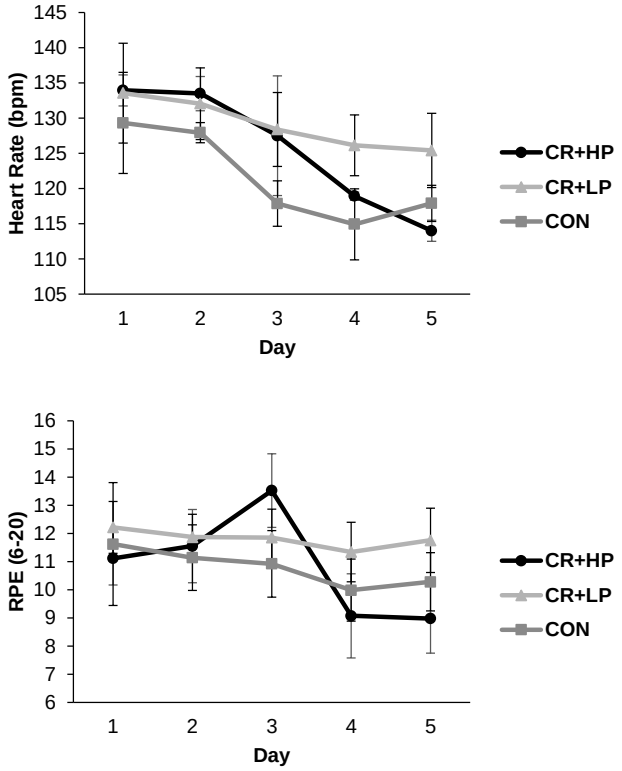

Figure 4.7: Changes in Heart Rate and RPE during prescribed exercise over the course of each 5-day intervention, consisting of caloric restriction (CR; 15 kcal/kg fat free mass (FFM)) with (+HP) or without (+LP) a high protein intake and normal energy availability (CON; kcal/kg FFM). Bars represent the group mean, and error bars represent the standard error of the mean.

Hormones

Insulin decreased by 53.6 pmol/L [-71.1; -36.2] during CR+HP (p = 0.05). Insulin did not change significantly during all other conditions. IGF-1 concentration decreased by 0.4 ng/mL [0.4; 0.0] during CR+LP (p = 0.05), but did not change during all other conditions. IGF-1BP concentration tended to decreased by 2.3 ng/mL [-11.4; 0.9] during CR+LP (p = 0.08) and by 2.3 ng/mL [-4.6; 0.1] during CON (p = 0.1). IGF-BP3 concentration did not change significantly during CR+HP.

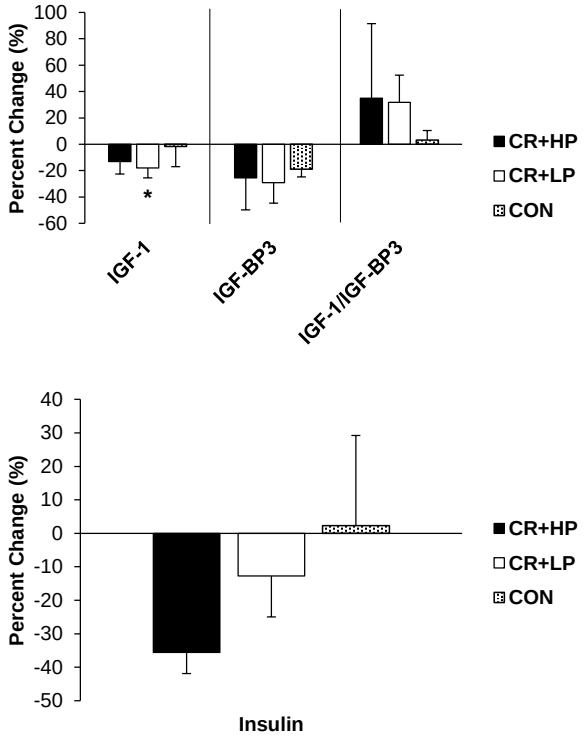

Figure 4.8: Percent changes in IGF-1, IGF-BP3, and insulin during prescribed exercise over the course of each 5-day intervention, consisting of caloric restriction (CR; 15 kcal/kg fat free mass (FFM)) with (+HP) or without (+LP) a high protein intake and

normal energy availability (CON; kcal/kg FFM. Bars represent the group mean, and error bars represent the standard error of the mean.

CHAPTER 5: DISCUSSION

The overall purpose of this thesis was to explore the capacity of exercise and a high protein intake to attenuate the side-effects of calorie-restricted weight loss. Two studies were utilized to address this purpose. The goals of study 1 were to determine the effects of a short-term, controlled energy deficit on measures of performance and exercise metabolism and explore whether exercise preserves the effects of a short-term, controlled energy deficit on these same outcomes. The goals of study 2 were to determine if an increased protein intake preserves FFM and aerobic performance during an energy deficit and determine if increased protein intake attenuates reductions of anabolic hormones (IGF-1, insulin, and testosterone).

Study 1:

The first purpose of study one was to determine the effects of controlled, short-term CR on measures of performance and exercise metabolism. Peak aerobic capacity and measures of strength did not change significantly in response to a 5-day CR where EA was reduced to 15 kcal/kg FFM/day. However, the 5-day CR resulted in increased heart rate and perceived exertion as well as elevated rates of fat oxidation during submaximal aerobic exercise along with impaired mood. The second purpose of study one was to explore whether changes in these outcomes could be attenuated by combining CR with exercise. When compared to CR-EX, CR+EX was associated with increased peak aerobic capacity, an attenuated loss of FFM such that weight loss consisted primarily of fat loss, and attenuated increases in heart rate and perceived exertion during submaximal exercise. Mood impairments were also prevented during CR+EX.

The preservation of peak aerobic capacity during both CR conditions was in agreement with a previous study in combat athletes [39], which found no changes in VO_{2max} following 7 days of CR. Contrary to these findings, another study reported a 8% reduction in maximal aerobic capacity after 5 days of CR in military personnel [7]. However, in this study, CR was combined with sleep deprivation, which may have affected endurance performance negatively. Individuals who are deprived of sleep are less motivated to endure discomfort and have increased perceived exertion levels during exercise, which may have led to an early cessation of an incremental test to exhaustion, thus resulting in a failure to achieve a true measure of VO_{2max} [90]. Another potential difference that could explain the discrepancy in the results is that Guezennec et al. failed to account for changes in body weight when reporting changes in VO_{2max}. In the current study, VO_{2max} was found to be maintained when expressed in absolute (L/min) or relative values (mL/kg/min). Unfortunately, no weight measures were reported by Guezennec et al. to verify the assumption that VO_{2max} normalized for changes in body weight may have been unaffected due to weight loss [7]. The participants in the current study were all aerobically trained and were performing aerobic exercise at least 3 hours per week prior to participation in the study. Since the CR-EX and CON-EX conditions required the participants to abstain from exercise for 4 days, detraining may have occurred during these conditions. However, detraining of 2-4 weeks is typically required to result in measurable reductions in VO_{2max}, which explains why VO_{2max} did not change during CR-EX or CON-EX [91]. The 6% increase in peak aerobic capacity during CR+EX was unforeseen due to the short duration of the study. The training stimulus of regular exercise may have promoted muscle protein synthesis to maintain muscle mass [92] and

mitochondrial density in the muscle [93], leading to the improvement in VO_{2max}. Past research found increases in VO_{2max} [18] when participants exercised during CR, but this increase was observed after a 12 week intervention in untrained overweight and obese older men and women. To our knowledge, this is the first instance of increased VO_{2max} during CR in a short-term study in trained individuals.

Unlike VO_{2max}, increases in heart rate and perceived exertion during submaximal exercise were expected during the CR-EX condition. Exercise attenuated the reduction of submaximal aerobic fitness during CR+EX as seen by the maintenance of heart rate and perceived exertion when compared to CR-EX. The constant training stimulus may have promoted a preservation of training status, resulting in a maintenance of effort required to perform the same power output [94]. Although not significant, CR+EX seemed to result in greater rates of fat oxidation when compared to CR-EX. Although our findings suggest that exercise attenuated the reductions in submaximal aerobic fitness during CR, CR has been shown to impair submaximal aerobic performance in past studies [5-7]. A 14% reduction in cycling time trial distance occurred after a 5-day intervention of CR in young male soldiers [7]. When testing competitive cyclist, time to fatigue was reduced by 15-64% after a 24 hour fast along with an increase in perceived exertion [5]. In young aerobically fit males, 48 hours of CR resulted in a 10.3% reduction in the distance covered during a 30 minute treadmill time trial [6]. To our knowledge, changes in submaximal performance have not been previously compared between CR with and without exercise.

Fat oxidation during submaximal exercise also increased during CR, suggesting that CR resulted in a shift to utilizing fat as a primary energy source to compensate for

the lack of calories consumed [95]. When performing 1-4 hours of aerobic exercise daily, it is suggested that individuals consume 7-12 g of carbohydrates per kg body weight to properly restore muscle glycogen stores between workouts [96]. When comparing the carbohydrate intake recommendations to the intake during the CR conditions, carbohydrate intake during CR+EX (3.38 g/kg) and CR-EX (1.69 g/kg) were both well below these recommendations. It should be noted that, although both CR conditions consumed well below the recommended amounts of carbohydrates, the intake CR-EX was only half of amount consumed during CR+EX and may have impacted submaximal aerobic performance to a greater extent. Increased fat oxidation was also previously been reported during isocaloric high fat diets and resulted in reduced endurance performance during short-term interventions [97-100].

As expected, strength and rate of force development were maintained during all conditions. This was in agreement with previous studies that found some measures of jump height did not change, but other measures of jump height increased during short-term, prescribed energy deficit interventions [10, 14]. The reduction in bench press strength during CR+EX is likely due to the absence of an exercise stimulus for the upper body. Unlike the leg extension and leg curl strength, which was maintained potentially due to the legs being stimulated during cycling [101], no exercise was performed to stimulate the muscles in the upper body [94]. The results of the bench press results are in agreement with a previous study which found decreased grip and back strength measures during short-term CR. The exercise that participants utilized to achieve the desired weight loss may have not adequately stimulated the muscle involved in the grip and back strength tests [12]. The loss of bench press strength suggests a need for direct exercise

stimulus to maintain strength in a particular limb/muscle group during an energy deficit, since CR may accelerate the negative effects of detraining [102]. More research is needed to determine the effect CR on strength measures and how limb specific exercises may prevent the loss of strength at various sites.

Our data indicate that mood was impaired during CR, and primarily during CR-EX, as shown by reductions in positive mood, self-confidence, sense of fitness, and physical energy. This is in agreement with previous studies showing increased fatigue [9], confusion, anger, and tension as well as decreased vigor during CR [13, 14, 21].

Study 2:

The first purpose of study 2 was to determine if an increased protein intake preserves FFM and aerobic performance during an energy deficit. When compared to low protein (0.8 g/kg), increasing the protein intake to 1.7 g/kg during CR preserved FFM, tended to increased peak aerobic capacity by 15.5 ± 4.2%, increased maximal fat oxidation by 87.8 ± 6.5% and reduced heart rate and perceived exertion during exercise training.

The second purpose of the second study was to determine if increased protein intake attenuates reductions of anabolic hormones (IGF-1,IGF-BP3, and insulin). During a high protein intake, insulin concentration tended to decrease by 35.6 ± 6.3%, and, unlike CR+LP, IGF-1 and IGF-BP3 did not significantly change.

The preservation of FFM during CR+HP was in agreement with a systematic review assessing the effects of caloric restriction and high protein intake in body composition, showing that a high protein intake (1.07-1.60 g/kg) attenuated the loss of

FFM and increased FM losses when compared to low protein intake (0.55-0.88 g/kg) [72]. A high protein diet works to preserve FFM by promoting increased levels of circulating amino acids, which activates signaling to stimulate increased MPS and thus skeletal muscle growth [4]. The increase in MPS during CR is supported by a study conducted by Hector et al. which showed that a high protein intake (1.3 g/kg) during 14 days of CR was found to attenuate the loss of MPS in overweight and obese men and women [22]. Positive effects on MPS were also observed when protein is consumed post-exercise. Consuming 15-30 g of protein during CR and after a single bout of resistance exercise resulted in elevated rates of MPS above resting values in an energy balance [15]. The effects of increased MPS as a result of a high protein intake during CR are supported by weight loss interventions utilizing aerobic exercise. A 6 month CR study in overweight and obese men split participants either a high protein group (1.2 g/kg) or a low protein group (0.8 g/kg). The high protein intake resulted in a maintenance of FFM, unlike the low protein intake which resulted in a loss of FFM [25]. It should be noted that, although the current study was shorter than previous studies, similar effects on the preservation of FFM were found. When comparing the results of study 1 and 2 on FFM, it is clear that the increased protein intake has additive effects on the maintenance of FFM. When incorporating the high protein intake, the loss of FFM decreased from -1.0 kg in the CR+EX condition in study 1 to -0.15 kg in the CR+HP condition in study 2. It should be noted that CR+LP (study 2) lost even more FFM (1.76 kg) and cannot be considered as a direct comparison to CR+EX, considering the protein intake during CR+EX (study 1) was almost twice (1.38 g/kg) when compared to CR+LP (study 2; 0.8 g/kg). Therefore, past literature agrees with our findings that a high protein intake

coupled with exercise will further promote favorable changes in body composition during CR.

The trend suggesting an increase in peak aerobic capacity and preservation of power at peak aerobic capacity is in partial agreement with study 1 and previous studies. As shown in study 1, performing aerobic exercise during CR attenuated the loss of aerobic performance and combining a high protein diet has been able to further protect performance to a greater extent than exercise alone. Although peak aerobic capacity increased during CR+EX, it did not during CR+LP. As suggested previously, the protein intake during CR+LP (study 2; 0.8 g/kg) was lower than CR+EX (study 1; 1.38 g/kg) and may explain why we did not see an increase in peak aerobic capacity. The increasing in protein intake amounts during study 1 and 2 highlight the effect it has on the preservation of aerobic performance, as shown by a 1.4% reduction in VO_{2peak} during CR+LP (study 2; 0.8 g/kg), a 6.2% increase in VO_{2peak} during CR+EX (study 1; 1.38 g/kg), and a 15.5% increase in VO_{2peak} during CR+LP (study 2; 1.7 g/kg). Since protein degradation is increased during CR, the increase of protein intake is able to increase protein synthesis and maintain or improves the muscle's ability to adapt to the mechanical stress of regular exercise [103]. The improved protein balance results in a maintenance of muscle mass and function, allowing adaptation to the exercise stimulus and improvement in aerobic performance, regardless of weight loss [15].

The increase in fat oxidation in CR+HP is supported by previous studies showing an increase in fat oxidation when a greater amount of protein is consumed. Fat oxidation was increased after a high protein breakfast was consumed in overweight children when compared to a low protein breakfast with similar caloric density [104]. A high protein

meal also increased fat oxidation in lean and overweight women when compared to a low protein meal with similar caloric content [105]. Although few studies have addressed the change in maximal fat oxidation when consuming a high protein diet during CR, it should be noted that a strong association exists between resting fat oxidation and maximal fat oxidation [106].

The reduction in insulin during the high protein intake agrees with the hypothesis of study 2 and is in agreement with previous studies, where insulin was suppressed during an energy deficit when consuming a high protein intake in obese individuals [22, 24]. The decrease in glucose consumption, which occurred as a result of the reduction in total calories consumed and the reduced ratio of calories consumed as carbohydrates to increase total protein content to the target levels, may have led to the reduced amount of circulating insulin. Also, the maintained FFM as a result of the high protein intake and exercise may have maintained or increased insulin sensitivity, which would reduce the need for large increases in insulin to adequately uptake glucose into the muscles.

The attenuation of the reduction of IGF-1 concentrations is in agreement with previous studies. Unfortunately, mixed results have been found regarding the effect of a high protein diet on IGF-1 during an energy deficit. A protein intake of 1.63-1.8 g/kg BW failed to prevent the reduction of IGF-1 in young men and women over the course of 12 days to 1 month of CR when compared to a protein intake of 0.8 g/kg BW [31-33], although a protein intake of 0.9 g/kg BW attenuated the reduction in IGF-1 during days 4-8 in young men during an 8-day energy deficit when compared to a protein intake of 0.5 g/kg BW [29, 30]. It should be noted that the study conducted by Rarick et al. did not have an exercise component and both studies that found a reduction in IGF-1

concentrations only had a 26.6-39.5% energy deficit, unlike the study by Alemany et al. and the current study which had a 58.8% and 62.5% energy deficit respectively. The effects of a high protein intake may be more effective during a short-term, severe energy deficit while exercising in preventing catabolism.

LIMITATIONS

Although study 1 was designed to assess the impact of controlled short-term CR on measures of performance and exercise metabolism and whether exercise can preserve these measures in response to caloric restriction, it failed to address certain topics. For example, the macronutrient distribution was similar during all conditions, resulting in a protein intake that was almost twice as high during CR+EX (1.38 g/kg) when compared to CR-EX (0.77 g/kg). Although the recommended daily allowance of protein intake for the general population is 0.8 g/kg body weight (BW), it has been suggested that a protein intake above this level may be beneficial during an energy deficit. The higher protein intake may have inadvertently improved the participant's ability to maintain muscle mass and thus performance [19], as suggested by findings that show a high protein diet (1.3-2.4 g/kg BW) can attenuate the reduction in muscle protein synthesis and either maintain or increase FFM during short-term CR interventions [19, 22-26]. In study 1, macronutrient content was kept at a standard ratio to avoid any effects due to changes in diet composition and to maintain a similar diet across conditions. The effects of different protein compositions during CR was not the focus of study 1, but was of interest for study 2 regarding the ability of a high protein intake to preserve FFM and provide

positive benefits for performance. As suggested by study 2, the increase in protein intake has additive effects on preservation of FFM and aerobic performance

Another aspect that was not addressed in neither study is the restoration of muscle glycogen upon refeeding. Reductions in performance during CR-EX may have been caused by a reduction in muscle glycogen due to reduced carbohydrate intake during CR, causing an increased utilization of fat stores as suggested by the increased fat oxidation. Refeeding participants after cessation of each condition may have restored glycogen stores and may have further attenuated reductions in submaximal performance [11, 13, 97-100]. Although refeeding the participants was not within the scope of this study, other researchers may want to restore glycogen stores after weight loss, specifically in athletes who participate in weight class sports and would be able to refeed prior to competition [6, 35, 107].

The present study focused on lab-related measures of performance and exercise metabolism. From an athlete's perspective, performance within competition may be the most important performance outcome due to its direct applicability. Previous studies on the effect of CR on athletic performances have shown mixed results. For example, females swimmers who exhibited signs of chronic CR experienced a reduction in swim velocity following 12 weeks of training [108]. Another study, found no change in sport-specific performance in judo athletes who restricted calories over 5-7 days [11]. Although we did not determine direct measures of athletic performance, our results are in agreement with previous studies, suggestion that submaximal aerobic performance is suppressed and strength and anaerobic performance are maintained, especially during CR+EX.

Study 2 was designed to assess the effects of a high protein diet on FFM, aerobic performance during CR, but there were some limitations of this study. Considering that 3 participants decided not to participate after completing 1 condition and only 3 participants completed all 3 conditions, an uneven amount of participants completed each condition. The uneven conditions resulted in a greater amount of participants to complete the low protein condition than the high protein condition, which may have impacted the comparison between conditions. Since this a pilot study, more data is needed to achieve an accurate comparison of conditions.

Although the high protein diet was able to preserve FFM and performance, RMR decreased by 209 kcals/day during CR+HP ($p < 0.01$), but not during all other conditions. The suppression of resting metabolic rate during CR+HP was unexpected, considering that FFM was maintained during this condition. One possible explanation for this occurrence is the impact of the thermic effect of food. Typically, the thermic effect of food increases the energy expenditure by 10% of the energy consumed, but the thermic effect of food can be 15% or more for a high protein diet [109]. When estimating the thermic effect of food for each condition, CR+HP expends about 119 kcals more than during CR+LP. Although the caloric content of the high and low protein diets were the same, the increased thermic effect of food may have contributed to a greater energy deficit, leading to a greater metabolic suppression separate from the effect of FFM [7, 110]. Although not controlled for in this thesis, I would suggest future studies to directly measure the thermic effect to of a high and low protein intake during CR and control for the thermic effect of food by adjusting the caloric intake of each condition so the energy deficits are equal.

The significant reduction of RMR during CR+HP may also be the result of participants not fully regaining all of their weight between conditions. The 2 participants who completed each condition started in the high protein group and gradually lost weight throughout the study, which resulted in participants being progressively lighter and had a lower resting metabolic rate at the start of each condition. Since resting metabolic rate was higher in the first condition (CR+HP), participants underwent a greater energy deficit during the CR+HP than when those same participants completed the other conditions. Thus, the greater energy deficit may have led to a greater suppression of resting metabolic rate. Since this a pilot study, more data is needed to achieve an accurate comparison of conditions to determine if a similar reduction of RMR occurs during CR+HP when more participants complete the entire study in a balanced order.

Although the current study was designed to assess the impact of controlled short-term CR on measures of aerobic performance and exercise metabolism and whether a high protein diet can preserve these measures in response to caloric restriction, it failed to address the impact on measures of strength. Considering the results of study 1, we would expect that measures of maximal force and rate of force development would be maintained during CR+EX, which is comparable to the CR+LP condition in study 2. Since resistance exercise was not performed during study 2, it would be unexpected to observe significant increases in strength measures. Longland et al. found no difference in the changes in push up, sit ups, ankle knee extension torque after a 4 week CR intervention consisting of either a low protein intake (1.2 g/kg) or a high protein intake (2.4 g/kg), however both protein intakes were well above recommended daily allowance for protein intake (0.8 g/kg) [26]. In a study by Josse et al., all groups improved in all

strength measures, however the high protein group (1.33 g/kg) had a tendency for greater strength in the chest press, hamstring curl, and seated row after 16 weeks of CR when compared to the low protein group (0.84 g/kg) The effect of a high protein diet on strength measures during CR was not the focus of our study, however it would be of interest to further determine if a high protein intake is able to increase strength measures during CR.

CONCLUSION

Our findings demonstrate that short-term CR, achieved by reducing EA to 15 kcal/kg FFM/day, leads to a loss of FFM and is associated with increased HR, RPE, and fat oxidation during submaximal aerobic exercise, as well as reductions in mood state; whereas aerobic performance and strength were maintained. The addition of exercise to short-term CR attenuated the reductions in HR, RPE, and mood state, and increased aerobic performance. Coupling aerobic exercise and a high protein diet during a short-term CR may be able to further preserve not only FFM but also measures of submaximal performance (e.g. HR, RPE) and anabolic hormones (IGF-1) and tended to increase aerobic performance. In summary, exercise, especially when combined with a high protein diet, seems to be an effective means to offset the negative effects of short-term CR on body composition, aerobic performance, and well-being.

CHAPTER 6: REFERENCES

1. Gault, M.L. and M.E. Willems, *Aging, functional capacity and eccentric exercise training.* Aging Dis, 2013. **4**(6): p. 351-63.
2. Fukushima, Y., et al., *Importance of Lean Muscle Maintenance to Improve Insulin Resistance by Body Weight Reduction in Female Patients with Obesity.* Diabetes Metab J, 2016. **40**(2): p. 147-53.
3. Weinheimer, E.M., L.P. Sands, and W.W. Campbell, *A systematic review of the separate and combined effects of energy restriction and exercise on fat-free mass in middle-aged and older adults: implications for sarcopenic obesity.* Nutr Rev, 2010. **68**(7): p. 375-88.
4. Guimarães-Ferreira, L., et al., *Synergistic effects of resistance training and protein intake: practical aspects.* Nutrition, 2014. **30**(10): p. 1097-103.
5. Loy, S.F., et al., *Effects of 24-hour fast on cycling endurance time at two different intensities.* J Appl Physiol (1985), 1986. **61**(2): p. 654-9.
6. Nieman, D.C., et al., *Running endurance in 27-h-fasted humans.* J Appl Physiol (1985), 1987. **63**(6): p. 2502-9.
7. Guezennec, C.Y., et al., *Physical performance and metabolic changes induced by combined prolonged exercise and different energy intakes in humans.* Eur J Appl Physiol Occup Physiol, 1994. **68**(6): p. 525-30.
8. Oliver, S.J., et al., *Endurance running performance after 48 h of restricted fluid and/or energy intake.* Medicine & Science in Sports & Exercise, 2006: p. 7.
9. Rome, E.S., *Eating disorders.* Obstet Gynecol Clin North Am, 2003. **30**(2): p. 353-77, vii.
10. Fogelholm, G.M., et al., *Gradual and rapid weight loss: effects on nutrition and performance in male athletes.* Med Sci Sports Exerc, 1993. **25**(3): p. 371-7.
11. Artioli, G.G., et al., *Rapid weight loss followed by recovery time does not affect judo-related performance.* J Sports Sci, 2010. **28**(1): p. 21-32.
12. Jlid, M.C., et al., *Rapid weight loss alters muscular performance and perceived exertion as well as postural control in elite wrestlers.* J Sports Med Phys Fitness, 2013. **53**(6): p. 620-7.
13. Degoutte, F., et al., *Food restriction, performance, biochemical, psychological, and endocrine changes in judo athletes.* Int J Sports Med, 2006. **27**(1): p. 9-18.
14. Filaire, E., et al., *Food restriction, performance, psychological state and lipid values in judo athletes.* Int J Sports Med, 2001. **22**(6): p. 454-9.
15. Areta, J.L., et al., *Reduced resting skeletal muscle protein synthesis is rescued by resistance exercise and protein ingestion following short-term energy deficit.* Am J Physiol Endocrinol Metab, 2014. **306**(8): p. E989-97.
16. van Gemert, W.A., et al., *Effect of weight loss, with or without exercise, on body composition and sex hormones in postmenopausal women: the SHAPE-2 trial.* Breast Cancer Res, 2015. **17**: p. 120.
17. Chomentowski, P., et al., *Moderate exercise attenuates the loss of skeletal muscle mass that occurs with intentional caloric restriction-induced weight loss in older, overweight to obese adults.* J Gerontol A Biol Sci Med Sci, 2009. **64**(5): p. 575-80.

18. Yoshimura, E., et al., *Aerobic exercise attenuates the loss of skeletal muscle during energy restriction in adults with visceral adiposity.* Obes Facts, 2014. **7**(1): p. 26-35.
19. Layman, D.K., et al., *Dietary protein and exercise have additive effects on body composition during weight loss in adult women.* J Nutr, 2005. **135**(8): p. 1903-10.
20. Cooney, G.M., et al., *Exercise for depression.* Cochrane Database Syst Rev, 2013(9): p. CD004366.
21. Karl, J.P., et al., *Altered metabolic homeostasis is associated with appetite regulation during and following 48-h of severe energy deprivation in adults.* Metabolism, 2016. **65**(4): p. 416-27.
22. Hector, A.J., et al., *Whey protein supplementation preserves postprandial myofibrillar protein synthesis during short-term energy restriction in overweight and obese adults.* J Nutr, 2015. **145**(2): p. 246-52.
23. Josse, A.R., et al., *Increased consumption of dairy foods and protein during diet- and exercise-induced weight loss promotes fat mass loss and lean mass gain in overweight and obese premenopausal women.* J Nutr, 2011. **141**(9): p. 1626-34.
24. Noakes, M., et al., *Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women.* Am J Clin Nutr, 2005. **81**(6): p. 1298-306.
25. Soenen, S., et al., *Normal protein intake is required for body weight loss and weight maintenance, and elevated protein intake for additional preservation of resting energy expenditure and fat free mass.* J Nutr, 2013. **143**(5): p. 591-6.
26. Longland, T.M., et al., *Higher compared with lower dietary protein during an energy deficit combined with intense exercise promotes greater lean mass gain and fat mass loss: a randomized trial.* Am J Clin Nutr, 2016. **103**(3): p. 738-46.
27. Levine, M.E., et al., *Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population.* Cell Metab, 2014. **19**(3): p. 407-17.
28. Harel, Z. and G.S. Tannenbaum, *Dietary protein restriction impairs both spontaneous and growth hormone-releasing factor-stimulated growth hormone release in the rat.* Endocrinology, 1993. **133**(3): p. 1035-43.
29. Alemany, J.A., et al., *Effects of dietary protein content on IGF-I, testosterone, and body composition during 8 days of severe energy deficit and arduous physical activity.* J Appl Physiol (1985), 2008. **105**(1): p. 58-64.
30. Rizzoli, R., et al., *Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly.* Bone, 2010. **46**(2): p. 294-305.
31. Cao, J.J., et al., *Calcium homeostasis and bone metabolic responses to high-protein diets during energy deficit in healthy young adults: a randomized controlled trial.* Am J Clin Nutr, 2014. **99**(2): p. 400-7.
32. Henning, P.C., et al., *High protein diets do not attenuate decrements in testosterone and IGF-I during energy deficit.* Metabolism, 2014. **63**(5): p. 628-32.
33. Rarick, K.R., et al., *Energy flux, more so than energy balance, protein intake, or fitness level, influences insulin-like growth factor-I system responses during 7*

days of increased physical activity. J Appl Physiol (1985), 2007. **103**(5): p. 1613-21.
34. Hill, J.O., *Understanding and addressing the epidemic of obesity: an energy balance perspective.* Endocr Rev, 2006. **27**(7): p. 750-61.
35. Donnelly, J.E., et al., *American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults.* Med Sci Sports Exerc, 2009. **41**(2): p. 459-71.
36. Carbone, J.W., et al., *Effects of short-term energy deficit on muscle protein breakdown and intramuscular proteolysis in normal-weight young adults.* Appl Physiol Nutr Metab, 2014. **39**(8): p. 960-8.
37. Bell, R.A., M. Al-Khalaf, and L.A. Megeney, *The beneficial role of proteolysis in skeletal muscle growth and stress adaptation.* Skelet Muscle, 2016. **6**: p. 16.
38. Forbes, G.B., *Body fat content influences the body composition response to nutrition and exercise.* Ann N Y Acad Sci, 2000. **904**: p. 359-65.
39. Reljic, D., et al., *Rapid body mass loss affects erythropoiesis and hemolysis but does not impair aerobic performance in combat athletes.* Scand J Med Sci Sports, 2016. **26**(5): p. 507-17.
40. Bar-Or, O., *The Wingate anaerobic test. An update on methodology, reliability and validity.* Sports Med, 1987. **4**(6): p. 381-94.
41. Shapses, S.A. and D. Sukumar, *Bone metabolism in obesity and weight loss.* Annu Rev Nutr, 2012. **32**: p. 287-309.
42. Lee, W.C., et al., *Energy Metabolism of the Osteoblast: Implications for Osteoporosis.* Endocr Rev, 2017.
43. Norman, A.W. and H.L. Henry, *Hormones*. 2014: Elsevier Science.
44. Dawson-Hughes, B., et al., *Implications of absolute fracture risk assessment for osteoporosis practice guidelines in the USA.* Osteoporos Int, 2008. **19**(4): p. 449-58.
45. Soltani, S., et al., *The effects of weight loss approaches on bone mineral density in adults: a systematic review and meta-analysis of randomized controlled trials.* Osteoporos Int, 2016. **27**(9): p. 2655-71.
46. Zibellini, J., et al., *Does Diet-Induced Weight Loss Lead to Bone Loss in Overweight or Obese Adults? A Systematic Review and Meta-Analysis of Clinical Trials.* J Bone Miner Res, 2015. **30**(12): p. 2168-78.
47. Khosla, S., et al., *Oral bisphosphonate-induced osteonecrosis: risk factors, prediction of risk using serum CTX testing, prevention, and treatment.* J Oral Maxillofac Surg, 2008. **66**(6): p. 1320-1; author reply 1321-2.
48. Iba, K., et al., *Changes in urinary NTX levels in patients with primary osteoporosis undergoing long-term bisphosphonate treatment.* J Orthop Sci, 2008. **13**(5): p. 438-41.
49. Broyles, D.L., et al., *Analytical and clinical performance characteristics of Tandem-MP Ostase, a new immunoassay for serum bone alkaline phosphatase.* Clin Chem, 1998. **44**(10): p. 2139-47.
50. Redman, L.M., et al., *Calorie restriction and bone health in young, overweight individuals.* Arch Intern Med, 2008. **168**(17): p. 1859-66.

51. Rigotti, N.A., et al., *Osteoporosis in women with anorexia nervosa.* N Engl J Med, 1984. **311**(25): p. 1601-6.
52. Koehler, K., et al., *Low energy availability in exercising men is associated with reduced leptin and insulin but not with changes in other metabolic hormones.* J Sports Sci, 2016. **34**(20): p. 1921-9.
53. Müller, M.J., et al., *Metabolic adaptation to caloric restriction and subsequent refeeding: the Minnesota Starvation Experiment revisited.* Am J Clin Nutr, 2015. **102**(4): p. 807-19.
54. Martin, B., et al., *Caloric restriction: impact upon pituitary function and reproduction.* Ageing Res Rev, 2008. **7**(3): p. 209-24.
55. Yakar, S., et al., *Circulating levels of IGF-1 directly regulate bone growth and density.* J Clin Invest, 2002. **110**(6): p. 771-81.
56. Douyon, L. and D.E. Schteingart, *Effect of obesity and starvation on thyroid hormone, growth hormone, and cortisol secretion.* Endocrinol Metab Clin North Am, 2002. **31**(1): p. 173-89.
57. Schwartz, M.W., et al., *Central nervous system control of food intake.* Nature, 2000. **404**(6778): p. 661-71.
58. Thrailkill, K.M., et al., *Is insulin an anabolic agent in bone? Dissecting the diabetic bone for clues.* Am J Physiol Endocrinol Metab, 2005. **289**(5): p. E735-45.
59. Breiner, M., G. Romalo, and H.U. Schweikert, *Inhibition of androgen receptor binding by natural and synthetic steroids in cultured human genital skin fibroblasts.* Klin Wochenschr, 1986. **64**(16): p. 732-7.
60. Misra, M. and A. Klibanski, *The neuroendocrine basis of anorexia nervosa and its impact on bone metabolism.* Neuroendocrinology, 2011. **93**(2): p. 65-73.
61. Ihle, R. and A.B. Loucks, *Dose-response relationships between energy availability and bone turnover in young exercising women.* J Bone Miner Res, 2004. **19**(8): p. 1231-40.
62. De Souza, M.J. and N.I. Williams, *Beyond hypoestrogenism in amenorrheic athletes: energy deficiency as a contributing factor for bone loss.* Curr Sports Med Rep, 2005. **4**(1): p. 38-44.
63. Loucks, A., *Energy balance and energy availability*, in *Sports Nutrition, The Encyclopaedia of Sports Medicine, and IOC Medical Commission Publication*, R. Maughan, Editor. 2013, John Wiley & Sons, Ltd West Sussex, UK. p. 72-87.
64. West, D.W., et al., *Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors.* J Appl Physiol (1985), 2010. **108**(1): p. 60-7.
65. Spangenburg, E.E., et al., *A functional insulin-like growth factor receptor is not necessary for load-induced skeletal muscle hypertrophy.* J Physiol, 2008. **586**(1): p. 283-91.
66. Hornberger, T.A., et al., *Mechanical stimuli regulate rapamycin-sensitive signalling by a phosphoinositide 3-kinase-, protein kinase B- and growth factor-independent mechanism.* Biochem J, 2004. **380**(Pt 3): p. 795-804.
67. Schoenfeld, B.J., *The mechanisms of muscle hypertrophy and their application to resistance training.* J Strength Cond Res, 2010. **24**(10): p. 2857-72.

68. Gonzalez, A.M., et al., *Intramuscular Anabolic Signaling and Endocrine Response Following Resistance Exercise: Implications for Muscle Hypertrophy.* Sports Med, 2016. **46**(5): p. 671-85.
69. Cava, E., N.C. Yeat, and B. Mittendorfer, *Preserving Healthy Muscle during Weight Loss.* Adv Nutr, 2017. **8**(3): p. 511-519.
70. Kelley, G.A., K.S. Kelley, and W.M. Kohrt, *Exercise and bone mineral density in premenopausal women: a meta-analysis of randomized controlled trials.* Int J Endocrinol, 2013. **2013**: p. 741639.
71. Shah, K., et al., *Exercise training in obese older adults prevents increase in bone turnover and attenuates decrease in hip bone mineral density induced by weight loss despite decline in bone-active hormones.* J Bone Miner Res, 2011. **26**(12): p. 2851-9.
72. Wycherley, T.P., et al., *Effects of energy-restricted high-protein, low-fat compared with standard-protein, low-fat diets: a meta-analysis of randomized controlled trials.* Am J Clin Nutr, 2012. **96**(6): p. 1281-98.
73. MacDonell, R., M.W. Hamrick, and C.M. Isales, *Protein/amino-acid modulation of bone cell function.* Bonekey Rep, 2016. **5**: p. 827.
74. Chevalley, T., et al., *High-protein intake enhances the positive impact of physical activity on BMC in prepubertal boys.* J Bone Miner Res, 2008. **23**(1): p. 131-42.
75. Loucks, A.B., B. Kiens, and H.H. Wright, *Energy availability in athletes.* J Sports Sci, 2011. **29 Suppl 1**: p. S7-15.
76. Hilton, L.K. and A.B. Loucks, *Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women.* American journal of physiology. Endocrinology and metabolism, 2000. **278**(1): p. E43-9.
77. Loucks, A.B. and J.R. Thuma, *Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women.* The Journal of clinical endocrinology and metabolism, 2003. **88**(1): p. 297–311.
78. Ihle, R. and A.B. Loucks, *Dose-response relationships between energy availability and bone turnover in young exercising women.* Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research, 2004. **19**(8): p. 1231-40.
79. Loucks, A.B., M. Verdun, and E.M. Heath, *Low energy availability, not stress of exercise, alters LH pulsatility in exercising women.* Journal of applied physiology, 1998. **84**(1): p. 37-46.
80. Loucks, A.B. and E.M. Heath, *Induction of low-T3 syndrome in exercising women occurs at a threshold of energy availability.* The American journal of physiology, 1994. **266**(3 Pt 2): p. R817-23.
81. Loucks, A.B., *Low energy availability in the marathon and other endurance sports.* Sports medicine, 2007. **37**(4-5): p. 348-52.
82. Westerterp, K.R., *Pattern and intensity of physical activity.* Nature, 2001. **410**(6828): p. 539.
83. Cunningham, J.J., *Body composition as a determinant of energy expenditure: a synthetic review and a proposed general prediction equation.* Am J Clin Nutr, 1991. **54**(6): p. 963-9.
84. Armstrong, L.E., *Assessing hydration status: the elusive gold standard.* J Am Coll Nutr, 2007. **26**(5 Suppl): p. 575S-584S.

85. Schneider, S., et al., *The effect of parabolic flight on perceived physical, motivational and psychological state in men and women: correlation with neuroendocrine stress parameters and electrocortical activity.* Stress, 2009. **12**(4): p. 336-49.
86. Minahan, C., M. Chia, and O. Inbar, *Does power indicate capacity? 30-s Wingate anaerobic test vs. maximal accumulated O2 deficit.* Int J Sports Med, 2007. **28**(10): p. 836-43.
87. Hoffer, L.J. and R.A. Forse, *Protein metabolic effects of a prolonged fast and hypocaloric refeeding.* Am J Physiol, 1990. **258**(5 Pt 1): p. E832-40.
88. Borg, G., *[Physical training. 3. Perceived exertion in physical work].* Lakartidningen, 1970. **67**(40): p. 4548-57.
89. WEIR, J.B., *New methods for calculating metabolic rate with special reference to protein metabolism.* J Physiol, 1949. **109**(1-2): p. 1-9.
90. Thun, E., et al., *Sleep, circadian rhythms, and athletic performance.* Sleep Med Rev, 2015. **23**: p. 1-9.
91. Neufer, P.D., *The effect of detraining and reduced training on the physiological adaptations to aerobic exercise training.* Sports Med, 1989. **8**(5): p. 302-20.
92. Harber, M.P., et al., *Muscle protein synthesis and gene expression during recovery from aerobic exercise in the fasted and fed states.* Am J Physiol Regul Integr Comp Physiol, 2010. **299**(5): p. R1254-62.
93. Lundby, C. and R.A. Jacobs, *Adaptations of skeletal muscle mitochondria to exercise training.* Exp Physiol, 2016. **101**(1): p. 17-22.
94. Garber, C.E., et al., *American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise.* Med Sci Sports Exerc, 2011. **43**(7): p. 1334-59.
95. Frank, P., et al., *Acute exercise reverses starvation-mediated insulin resistance in humans.* Am J Physiol Endocrinol Metab, 2013. **304**(4): p. E436-43.
96. Burke, L.M., B. Kiens, and J.L. Ivy, *Carbohydrates and fat for training and recovery.* J Sports Sci, 2004. **22**(1): p. 15-30.
97. Burke, L.M., et al., *Effect of fat adaptation and carbohydrate restoration on metabolism and performance during prolonged cycling.* J Appl Physiol (1985), 2000. **89**(6): p. 2413-21.
98. Burke, L.M., et al., *Adaptations to short-term high-fat diet persist during exercise despite high carbohydrate availability.* Med Sci Sports Exerc, 2002. **34**(1): p. 83-91.
99. Burke, L.M. and J.A. Hawley, *Effects of short-term fat adaptation on metabolism and performance of prolonged exercise.* Med Sci Sports Exerc, 2002. **34**(9): p. 1492-8.
100. Havemann, L., et al., *Fat adaptation followed by carbohydrate loading compromises high-intensity sprint performance.* J Appl Physiol (1985), 2006. **100**(1): p. 194-202.
101. Hulston, C.J., et al., *Protein intake does not increase vastus lateralis muscle protein synthesis during cycling.* Med Sci Sports Exerc, 2011. **43**(9): p. 1635-42.

102. Mujika, I. and S. Padilla, *Detraining: loss of training-induced physiological and performance adaptations. Part I: short term insufficient training stimulus.* Sports Med, 2000. **30**(2): p. 79-87.
103. Pasiakos, S.M., H.R. Lieberman, and T.M. McLellan, *Effects of protein supplements on muscle damage, soreness and recovery of muscle function and physical performance: a systematic review.* Sports Med, 2014. **44**(5): p. 655-70.
104. Baum, J.I., M. Gray, and A. Binns, *Breakfasts Higher in Protein Increase Postprandial Energy Expenditure, Increase Fat Oxidation, and Reduce Hunger in Overweight Children from 8 to 12 Years of Age.* J Nutr, 2015. **145**(10): p. 2229-35.
105. Gentile, C.L., et al., *Resistant starch and protein intake enhances fat oxidation and feelings of fullness in lean and overweight/obese women.* Nutr J, 2015. **14**: p. 113.
106. Robinson, S.L., et al., *Maximal fat oxidation during exercise is positively associated with 24-hour fat oxidation and insulin sensitivity in young, healthy men.* J Appl Physiol (1985), 2015. **118**(11): p. 1415-22.
107. Rankin, J.W., J.V. Ocel, and L.L. Craft, *Effect of weight loss and refeeding diet composition on anaerobic performance in wrestlers.* Med Sci Sports Exerc, 1996. **28**(10): p. 1292-9.
108. Vanheest, J.L., et al., *Ovarian suppression impairs sport performance in junior elite female swimmers.* Med Sci Sports Exerc, 2014. **46**(1): p. 156-66.
109. Pesta, D.H. and V.T. Samuel, *A high-protein diet for reducing body fat: mechanisms and possible caveats.* Nutr Metab (Lond), 2014. **11**(1): p. 53.
110. Rosenbaum, M. and R.L. Leibel, *Adaptive thermogenesis in humans.* Int J Obes (Lond), 2010. **34 Suppl 1**: p. S47-55.