EFFECT OF DIETARY PROTEIN INTAKE ON BODY COMPOSITION CHANGES DURING INTENSE TRAINING IN AN ENERGY DEFICIT

EFFECT OF DIETARY PROTEIN INTAKE ON BODY COMPOSITION CHANGES DURING INTENSE TRAINING IN AN ENERGY DEFICIT

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ABSTRACT

Higher dietary protein intake, greater than the Recommended Dietary Allowance (RDA; 0.8 g protein/kg/d), coupled with resistive exercise has been shown to aid in preservation of muscle mass during hypocaloric diet-induced weight loss. We examined the impact of dietary protein supplementation at two levels (1.2 and 2.4 g/kg/d) on body composition during a 4wk hypocaloric dietary intervention that included 6d/wk of high intensity resistance exercise and interval training. In a single blind study, forty young men consumed 33±1.1 kcal/LBM (~40% reduction versus estimated energy requirements), and were randomly assigned to a group that consumed either 1.2g/kg/d protein or 2.4g/kg/d. Body composition was determined using DXA, Bod Pod, and Bio-impedance pre- and post-intervention to derive a 4-compartment model for body composition. Both groups retained lean mass (LM), but retention was greater in the higher protein group ($p<0.05$). Furthermore, the higher protein group had a greater loss of fat mass (FM) ($p<0.05$). Measures of exercise performance improved equivalently as a result of training with no effect of protein supplementation ($p<0.05$). A battery of tests designed to evaluate psychological measures, cognition, and executive function showed effects for training, in some cases, but no difference between groups. Our results show that consumption of 2.4 g protein/kg/d with strategically timed protein consumption was more effective than consumption of a diet containing 1.2 g protein/kg/d in preserving lean mass during a 4wk period of substantial

energy restriction while performing a high volume of intense exercise. The effect of higher protein on mental performance during this same period appears limited.

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LIST OF ABBREVIATIONS

RDA – Recommended daily allowance
DXA – Dual x-ray absorptiometry
BIA – Bioelectrical impedance
LM – Lean mass
FM – Fat mass
MPS – Muscle protein synthesis
MPB – Muscle protein breakdown
NPB – Negative protein balance
FFM – Fat-free mass
1RM – One repetition maximum
REE – Resting energy expenditure
HIIT – High intensity interval training
HIIE – High intensity intermittent exercise
RT – Resistance training
QOL – Quality of life
LBM – Lean body mass
PRO – high protein (2.4 g/kg/d)
CON – normal protein (1.2g/kg/d)
BMI – Body mass index
BF – Body fat
MVC – Maximal voluntary contraction
PANAS – Positive and negative affect scale
POMS – Profile of mood states
OSPAN – Operation span test
OCT – Optimal cutting temperature (medium for tissue embedding)
RPM – Revolutions/min
CHO – Carbohydrates
4C – Four compartment body composition model
TBV – Total body volume
BMC – Bone mineral content
TBW – Total body water
ACSM – American college of sports medicine
WAT – Wingate anaerobic test
PBS – Phosphate buffered saline
PBST – Phosphate buffered saline with 2% tween
BSA – Bovine serum albumine
FBS – Fetal bovine serum
MCHI – Myosin heavy chain I
MCHII – Myosin heavy chain II
CSA – Cross-sectional area
SART – Sustained attention response task

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CHAPTER I

LITERATURE REVIEW

1. PROTEIN AND WEIGHT LOSS

While there is no doubt that the relative energy deficit created by dietary energy restriction and/or increased energy expenditure is the primary driver of weight loss, there is evidence that patterns of macronutrients provide flexibility for a more efficacious approach to weight loss. In this regard, a metabolically more beneficial strategy than generic scale-based 'weight loss' is one that focuses on 'quality' of weight loss. An improved 'quality' of weight loss is defined as being the loss of weight with the highest possible ratio of fat to lean mass loss. For an athlete such a pattern of weight loss may be especially relevant since preservation of lean mass may contribute to maintenance of performance. Several lines of evidence now suggest that increased dietary protein may be a beneficial macronutrient to consume in promoting quality weight loss.

1.1 Protein Turnover

The regulation of skeletal muscle mass is a dynamic process resulting in rapid turnover of proteins of ~280g/day in a 70kg man (1). The retention or loss of muscle protein mass occurs when there is an imbalance in the processes of muscle protein turnover. Muscle protein balance underpins muscle protein mass and is defined by the algebraic difference between muscle protein synthesis (MPS), and muscle protein breakdown (MPB). Multiple variables such as nutrition, exercise, age, and disease can determine the amount of time skeletal muscle mass spends in positive or negative protein balance (NPB). For example, if protein breakdown exceeds synthesis for transient periods such as during

immobilization, a net loss of skeletal muscle of ~40-60g/day occurs for a sedentary person weighing ~70-90kg (1).

In skeletal muscle, proteins are constantly being made from (synthesized), and degraded to, amino acids. The resultant protein turnover acts as a replacement for potentially damaged and dysfunctional proteins (2), while maintaining protein mass. However, when the availability of amino acids is increased through intravenous amino acid infusion or via amino acid/protein ingestion, rates of protein synthesis are markedly increased, with a small reduction in the rate of protein breakdown (3). The consequence is a positive NPB, or net anabolism in the skeletal muscle. Thus, amino acid availability appears to be an important stimulus regulating fluxes in MPS at rest. In healthy individuals, MPB is usually equal to MPS, resulting no net change in muscle mass, or size. When individuals are in the fed-state, periods of positive protein balance transpire. On the contrary, in the fasted-state periods of negative protein balance occur, generally equaling the positive gains experienced as a result of feeding as shown in Figure 1.

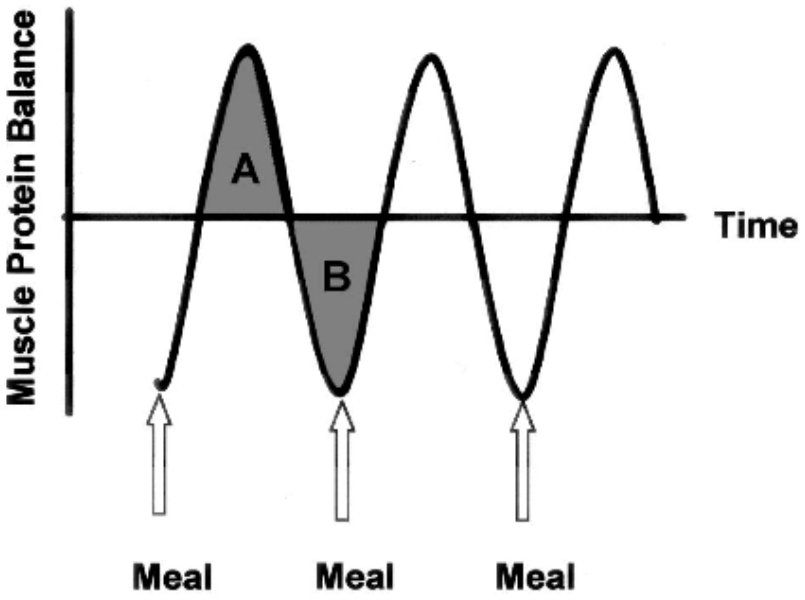

Figure 1: Fed and fasted-state changes in skeletal muscle protein balance. Area under the curve is for fed-state (A) is equivalent to area under the curve for fasted-state (B); from (4).

1.2 Nutritional Strategies for Weight Loss

Energy restricted diets are a common strategy for weight loss. Frequent macronutrient percentages of these diets include ~10-15% from protein, ~45-65% from carbohydrates, and <25-30% from fat (5); however, the 'optimal' macronutrient ratio for weight loss is a matter of debate. A recent meta-analysis from Wycherly et al. (5) and meta-regression from Krieger et al. (6) suggests that protein plays an important role in attenuating diet-induced reductions in fat free mass (FFM). The preservation of FFM may also have an impact on attenuating declines in resting energy expenditure during energy restriction. Higher protein diets (25-35% of energy intake) have been shown to increase body fat mass loss (5-7), and preserve FFM (5-7) during caloric restriction. However, diets lower in

carbohydrate, possibly as an exchange for protein, regardless of energy intake, seem to have an effect as well, garnering greater losses in body mass, greater losses in percent body fat, and greater losses of fat mass than diets with >40% energy from carbohydrates (6). Krieger et al., (6) report in this meta-regression that high-protein and low-carbohydrate diets have a positive effect toward what was referred to earlier as 'high quality' weight loss, independently of total energy intake.

Some variables, other than the absolute amount of protein intake, are important to maintain FFM during weight loss. For instance one such variable is the availability of the type of amino acids needed to maximize anabolic stimulation of skeletal muscle which is primarily driven by the digestibility and quality of the protein. The rapid aminoacidemia of a rapidly-digested protein, such as whey, stimulates muscle amino acid uptake to a greater extent than a slower release protein such as casein (4, 8). However, casein proteins attenuate whole-body proteolysis, which may result in a greater attenuation of muscle protein breakdown than whey (4, 8); in spite of this, the concordance between whole body and muscle proteolysis has never been demonstrated. Whey and casein are consumed together when ingesting milk, their ratio being ~4:1 casein to whey (4). Studies have reported that when compared to an isonitrogenous and nutrient-matched quantity of soy protein, milk supported greater protein anabolism in peripheral tissues (skeletal muscle) than soy (9, 10). The content of the amino acids within the protein, especially leucine, is also very important. High

quality, leucine-rich proteins will stimulate the most robust increase in MPS (11, 12). The potency of milk in comparison to soy in stimulating MPS rates can be partially attributed to higher levels of leucine. When looked at as a whole, available data suggests that skeletal muscle seems to be particularly sensitive to a leucine-rich protein, such as whey (11, 12).

Recent research from our lab showed that the amount of protein needed to create a robust stimulation of MPS in young persons can be as little as 10g, but maximal stimulation occurred at a protein dose of ~20g (11, 13). More recent analysis of these data (11, 13) has shown optimal dosages set at to be ~0.25-0.3g/kg/meal (D. Moore, manuscript in review). Current recommendations for protein intake are set by the recommended dietary allowance (RDA) at 0.8g/kg/d, but for athletes a published position stand sets protein intakes for resistance training athletes at 1.7-1.8 g/kg/d. (14). However, a recent pseudo-meta-analytical review suggested that protein intakes as high as 3.1g/kg/d (15) are recommended in those trying to lose weight while retaining lean mass. Obviously more research needs to be done to refine these numbers for athletes, and individuals with high-energy outputs, or low energy intakes looking to build or preserve FFM. Moreover, it appears higher protein intakes while at an energy deficit can help result in a 'higher quality' of weight loss. For example, Mettler et al., compared young men undergoing resistance training while consuming protein intakes of 1.0g/kg/d and 2.3g/kg/d of protein during a 40% reduction in energy intake over a two week period (7). The results showed a higher retention of lean

mass, and higher loss of fat mass in the higher protein group; thus, the increased provision of protein during energy restriction appears to help preserve lean mass and promotes fat mass loss.

Recent evidence suggests that habitual high protein consumption leads to an increased MPS response to a protein-containing beverage during an energy deficit (ED) (16). In addition, a recent study comparing protein consumption at the RDA to 2- and 3-times the RDA, showed that MPS increased in response to a Nestle BOOST drink only for the 3-times RDA group in the postprandial state during ED (Figure 2) (16). These findings indicate that increased habitual protein intake during energy deficit may have allowed enough amino acids following protein ingestion available for peripheral tissues such as muscle to result in a stimulation of MPS and sparing of lean mass (16).

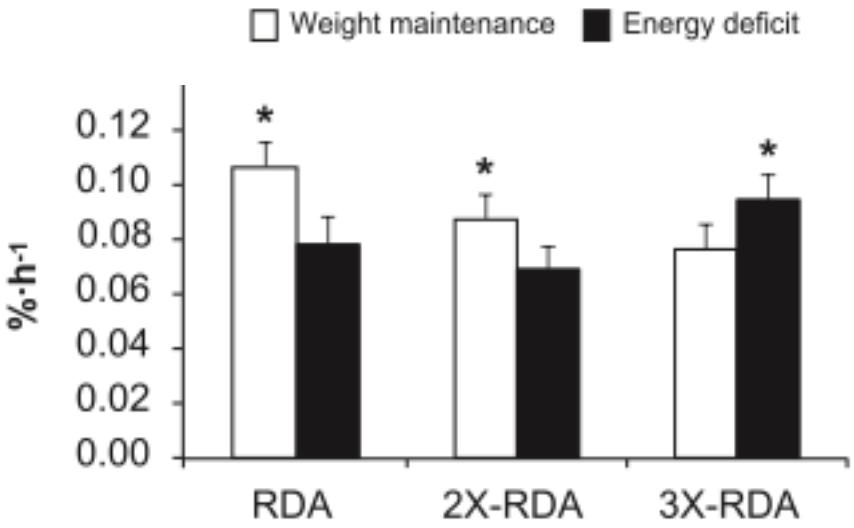

Figure 2: Skeletal muscle protein synthetic rates following feeding during weight maintenance and ED; from (16)

2. Resistance Training and Lean Mass

Resistance exercise is potent stimulator of skeletal MPS (17) and is an effective stimulus to improve muscle protein balance (18). In order to retain lean mass and induce hypertrophy, MPS needs to meet or exceed breakdown. However, Phillips et al. (18) showed that even in the fasted state the magnitude of the fasted-state negative protein balance was reduced due to MPS remaining elevated resulting in a more positive fasted-state NPB for up to 48hrs after a single bout of resistance exercise (Figure 3). Thus, resistance exercise creates longer durations of positive NPB during the post-absorptive state and results in lesser negative NPB while fasted due, for the most part, to a strong stimulation of MPS, resulting in lean mass accretion or hypertrophy.

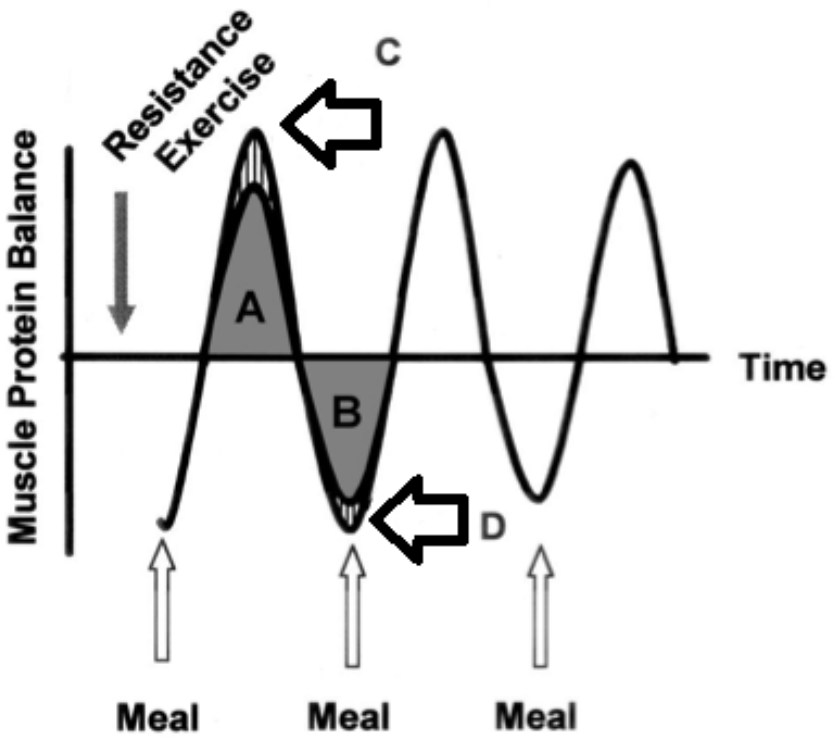

Figure 3: A. Fed-state gains in skeletal muscle protein balance. B. Fasted-state losses in skeletal muscle protein balance. C. Increases in fed-state gains with resistance exercise. D. Decreases in fasted-state losses with resistance exercise; from (4).

2.1 Exercise Intensity

It is a commonly held position that 'high-loads' are a necessity in resistance exercise programs, as they will result in greater muscle hypertrophy. However, a recent study by Burd et al. (19) challenged this notion by looking at a protocol employing high and low intensity contractions but performed to fatigue. According to Henneman et al. (20, 21) and the theory of orderly recruitment of motor units so long as fatigue (voluntary exhaustion) is achieved it means that full fibre activation has occurred. Utilizing higher loads when performing resistance training would increase the recruitment of muscle fibres, but there is a similar fibre recruitment when lower intensity contractions are performed until volitional fatigue or failure. Both methods of training have higher muscle fibre recruitment, exceeding the threshold for stimulating myofibrillar protein synthesis as shown in Figure 4 (20). Results from our lab have shown that when comparing a heavy intensity (90% 1RM), low volume (5±1 repetitions) resistance regimen, with a low-intensity (30% 1RM), higher volume (24±3 repetitions) resistance regimen, the lower intensity had a longer lasting effect on MPS rates (21-24hr) (19). The additional benefit seems to come from the additional number of repetitions, resulting in full motor unit recruitment (20). The longer-term implications of these results were borne out by a study performed by Mitchell et al, (22) who showed

that young men who trained with a load of ~30% of their 1RM experienced equivalent hypertrophy as those performing work at 80% of 1RM; however, voluntary isotonic strength gains were lower in the group training at 30% of 1RM. Thus, if maximal strength is the goal of the exercise regimen, higher intensity is superior (%1RM), as it serves as a more effective 'practice' for the muscle fibres in adjusting to the heavy load of a maximal lift (20, 22). However, lighter loads can be used effectively to stimulate MPS and, one would hypothesize, promote lean mass retention.

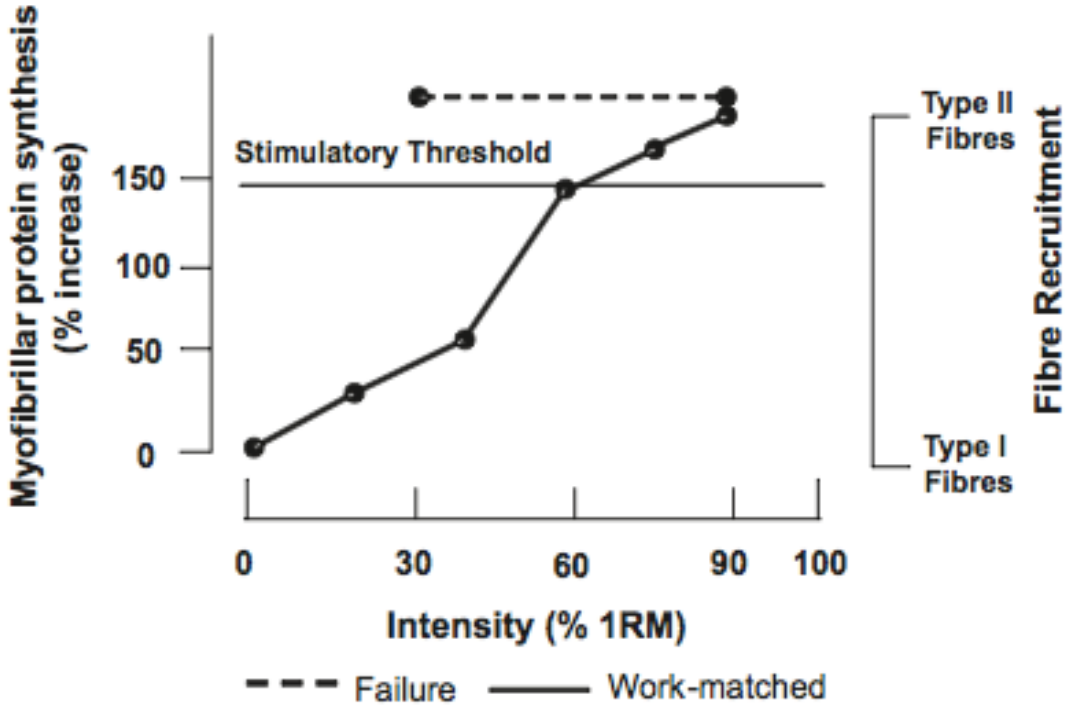

Figure 4: Resistance exercise intensity (x-axis) versus myofibrillar protein synthesis (y-axis). Bold line represents does-dependent relationship between resistance exercise intensity and myfibrillar protein synthesis rates; from (20)

2.2 Training While in an Energy Deficit

Data on improvements in exercise performance while weight is rapidly being lost is scarce, no improvement in performance seems to be the predominant finding (7, 23, 24). Although it is unclear why this is the case when subjects continue to train during the energy deficit; one speculation is that retention of lean mass might be a critical factor. When looking to retain lean mass, and increase fat mass loss in an energy deficits, high-fat, high protein diets are associated with greater retention of lean mass and losses of fat mass, respectively (5, 6). However, when training at high intensities, a lower carbohydrate diet can be detrimental to performance and training adaptations, not allowing athletes to train at the higher intensities desired (23-25). Throughout an athletic training cycle there may be advantages to either a low-carbohydrate, or low-fat diet, but having a higher protein intake to retain lean mass throughout remains beneficial to the athlete (1, 5, 15, 16).

2.3 Resistance Training in an Energy Deficit

Forbes' theory states that when an individual is in a state of caloric restriction, the loss of fat mass (~75%) will increase the risk of FFM (~25%) losses (26). FFM has a higher metabolic activity than fat, and its turnover is driven by skeletal muscle protein synthesis. A decrease in FFM lowers resting energy expenditure (REE), making the magnitude of energy deficit even smaller unless adjusted for

the expected decline in REE. Countermeasures to the loss of muscle mass, such as resistance training, have been shown to attenuate FFM losses by stimulating MPS, resulting in no net loss of FFM or even a gain in FFM (27). For example, Kraemer et al., altered the percentage weight loss by adding resistance training to an energy-restricted diet (27). With normal percentages of fat and lean mass losses during energy deficit being ~75 and 25%, respectively, adding just 3 days of heavy resistance training changed the percentages to 97% fat and 3% lean mass loss, with a high preservation of lean mass (27). This indicates that resistance training provides a unique stimulus while at an energy deficit to create positive NPB (27). A meta-analysis by Weinheimer et al. (28) reported that protein and resistance exercise independently help offset the normal hypocaloric diet-induced FFM losses.

2.4 Resistance Training Combined with Nutrition

It has been well established that amino acid and/or protein supplementation post resistance exercise stimulates MPS rates synergistically versus either stimulus alone (4, 13, 29-31). The hyperaminoacidemia with feeding post-exercise results in greater rates of MPS, and greater positive NPB than just amino acid ingestion at rest (Figure 5) (3). While there is some debate as to the timing of protein ingestion post exercise to induce a maximal synergistic stimulus, it appears that the earlier the post-exercise recovery process can start, the better (1). For example, in elderly men, a 2hr delay in protein provision after resistance exercise

was compared with ~5 min delay by Esmarck et al.(32). There were significant hypertrophic gains found in the early supplemented group (32), while the delayed group saw a decrease in mean fibre area and had reduced strength gains (32). This finding has not been reproduced, however, and others have not found the existence of such a short 'anabolic window'. For example, Burd et al. (33) showed that even 24h after performance of resistance exercise that MPS was still enhanced versus a fed-only condition. In fact, when the MPS response seen acutely post-exercise from the same study (19) is compared to that seen at 24h (33) then the magnitude of enhanced sensitivity of MPS was approximately the same. From an applied perspective, a recent meta-analysis of studies in which the timing of post-exercise supplementation has been compared in terms of supporting greater hypertrophy and/or strength gains also found no greater effect of immediate versus delayed post-exercise nutrition (primarily protein) consumption (34). Thus, while there appears to be no early post-exercise 'anabolic window' pragmatic advice would still be to provide nutrient support for recovery at the earliest possible time to augment as much of an adaption as possible (1).

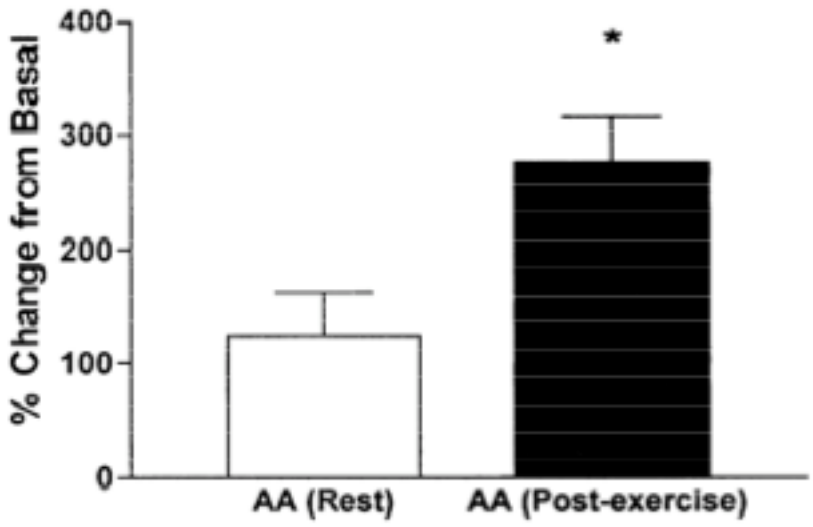

Figure 5: Rates of protein synthesis at rest and after exercise; from (3).

3. High Intensity Interval Training

There is a growing body of evidence to show that high intensity interval training (HIIT) is a time-efficient strategy to induce similar physiological adaptations to those that are normally associated with endurance training (35). Data also suggests that HIIT is an exercise modality that is associated with long term adherence (35). HIIT has been shown to significantly increase muscle oxidative capacity in only a two-week period, which is associated with improvement of the oxidation of fats and reduced risk for development of insulin resistance (35-37). Furthermore, HIIT is known to increase antioxidant enzyme activity, stimulate mitochondrial biogenesis, increase rates of MPS (especially in males), reduce the rate of glycogen utilization, and decrease lactate production during energy-matched work (35, 38, 39). As such it would seem prudent to include HIIT in a

program in which rapid adaptations in fitness and musculoskeletal oxidative capacity are being sought.

3.1 HIIT, Lean Mass Retention and Fat Loss

When looking at longer term effects of HIIT on lean mass retention and fat mass losses, Trapp et al., used a 15-week intervention of a 8s on 12s off cycling protocol for up to twenty minutes three times a week in females and showed a greater reduction in central abdominal fat mass compared to lower-intensity steady-state exercise and control groups (Figure 6) (40).

In men undertaking HIIT, Heydari et al (41) found that the same protocol used previously (40) for 20 min three times per week yielded similar results as abdominal fat mass dropped significantly by 2.0kg (41), which was significant when compared to a non-exercising control group. Interestingly, the same investigators also reported that FFM increased in the leg and trunk areas (41). There were also significant (15%) increases in VO_{2peak} in the HIIT intervention group (41). By inducing not only a preservation but potentially allowing for the gain of additional FFM, thus increasing the amount of metabolically active tissue, HIIT can likely be used to promote 'high quality' weight loss during energy deficit.

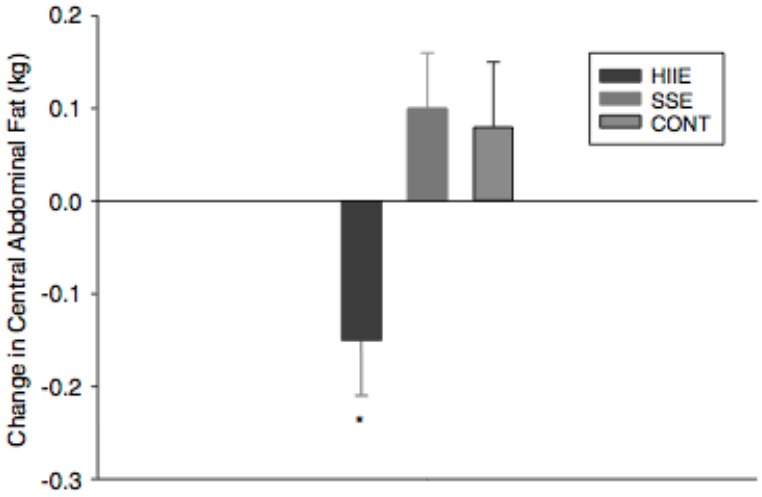

Figure 6: Change in central abdominal fat change for high-intensity intermittent exercise, steady-state exercise, and no exercise control group; from (40).

3.2 HIIT During an Energy Deficit

The resting rate of MPS is reduced during an energy deficit (42, 43), which would appear to result in a greater propensity for situations where net protein balance (NPB) was negative. Interventions such as resistance training and HIIT could be implemented to stimulate MPS and force NPB in a more positive direction. Recent work from Sartor et al., using a 14d diet-exercise study used two intervention groups who were consuming an energy restricted diet (25% daily deficit) with one group under diet-alone conditions and the other performed HIIT three times a week (36). Exercise consisted of up to ten 4min bouts of HIIT at 90% VO_{2peak} with 2-3 min rest on a cycle ergometer (36). Performance of HIIT successfully maintained FFM during this short-term energy deficit, whereas lean mass was lost in the diet-only group (36); thus, when looking to preserve lean

mass and attain 'high quality' weight loss, HIIT seems to be a promising exercise modality. In the same study HIIT also increased VO_{2peak} by 16% during the two-week intervention (36), a finding that is supported by a study by Talanian et al (37) who reported that VO_{2peak} increased by 12% with two weeks of HIIT in women.

If energy restriction is the predominant weight loss strategy, HIIT added to this regimen seems to promote a higher quality weight loss resulting in decreased (much like resistance training) losses of FFM, and increasing fat mass losses. While speculative, it would appear that the increased recruitment of muscle fibres at high enough loads to induce increases in MPS (44) during a bout of HIIT, would be a major factor in its potency in terms of preserving FFM (35). An added 'benefit' of HIIT training appears to be an improved aerobic fitness and added endurance capacity, which only serve to add to the attractiveness of this exercise modality.

3.3 HIIT and Nutrition

The stimulus of performing HIIT causes a stimulation of MPS that appears to be augmented by post-exercise nutrition (44), much like what is seen with resistance training (30). With the uncertainty of an 'optimal' anabolic window, the practical approach seems to be to consume fuel, containing some carbohydrate and protein, as soon as possible after exercise to take advantage of the anabolic stimulus of the exercise and the sensitivity of the muscle to hyperaminoacidemia

(1). With the high level of muscle activation and extensive muscle fibre recruitment involved with HIIT and RT, ingesting dietary protein post-exercise would result in immediate rebuilding and remodeling of the tissue, preparing the muscle to resume training at a high-intensity the subsequent day (44). However, a recent study conducted by Thomson et al., concluded that it is enhanced MPS recovery rates that increase later performance (45). The findings from Thomson et al., (45) agree with the increased rates of mixed muscle protein synthesis found in response to intense endurance exercise seen by Howarth et al., (45, 46). Thus, there may be a performance-enhancing aspect to protein provision that is related to stimulation of MPS, but this remains speculative. It also appears that the ingestion of dietary protein in close temporal proximity to the completion of exercise following HIIT results in an enhanced MPS response that could potentially lead to lean mass accretion while at an energy deficit.

4. Psychological State

Both exercise and energy restriction cause changes in a variety of psychological variables. Most research suggests that while exercise can have a beneficial effect on mood and reduce anxiety that energy restriction, particularly if marked, can have the opposite effect.

4.1 Mood in an Energy Deficit

Caloric restriction is an effective way to induce weight loss, resulting in a better quality of life (QOL), and improved mood (47). The difficulty, however, is in measuring the QOL and mood for the duration of the caloric restriction. Different methods to measure QOL and mood, usually in a questionnaire form, have yielded highly variable results. Redman et al., (47) conducted a six-month energy restriction trial, having participants ingest ~75% of their required energy via caloric restriction alone or caloric restriction and exercise. They reported that depressed mood, decreased QOL, or cognitive impairment was not associated with the intervention (47). However, cognitive impairment is associated with caloric restriction and one's preoccupation with food and body weight (48). This finding suggests that cognitive performance may be influenced by the 'obsessive' thoughts about food and weight during weight loss (48). However, data from Bryan et al., (49) suggests otherwise. These authors found that in a 12-week weight reduction study there were minimal effects on cognitive performance; however, if caloric restriction is severe then this may not be true. For example, in the Minnesota 'starvation experiment' (50) 36 healthy young men subjected themselves to a six-month starvation period of ~1800kcal/day and lost >25% of their body weight (51). Participants developed an obsession with food in this trial and experienced decrease in mood and cognitive function (50, 51). Obviously, the severity of the caloric restriction appears to be a major contributing factor to mood and cognitive functioning.

Green et al., (52) have suggested that it is spontaneous dieting, rather than caloric restriction, that results in cognitive impairment. These authors suggest that executive function is impaired because proposed thoughts of body image and food place demands on cognitive resources, limiting performance on cognitive tasks (52, 53). Thus, the effects on cognitive function may be related to a person's desire to lose weight versus simply having caloric restriction imposed upon them.

4.2 Mood and Exercise

With or without conscious awareness, the human body tries to sustain good moods and one technique used to regulate mood is through exercise (54). There is strong agreement that physical activity positively benefits mood (54-59). In normal populations exercise has been reported as the most effective behavioral technique used to self-regulate mood disturbances, leading to an increase in energy, reduction in feelings of stress, and alleviating negative mood states (54). An important effecter of changes in mood is the intensity of the exercise. A recent meta-analysis by Reed et al (59) shows that exercise intensity is generally negatively correlated with positive affect, thus leading them to prescribe an aerobic program that is optimal for improving positive affect at a low intensity (~30% VO_2), for 30-35min. Furthermore the duration of these programs seem to have the largest improvements in PA over 10-12 weeks suggesting that effects of PA are more difficult to discover in shorter acute studies (59). Although Reed et

al (59) calls this 'theory driven research' as the literature only leads to theoretical approaches to the effects of PA. To our knowledge most studies look to research the mood states of the clinical populations with mood disorders or older populations (61, 62), or children/adolescents (63), and the current study looks at healthy males 19-30yrs. Although, a similar study by Mettler et al (7) in athletes has looked at mood states while at an energy deficit with resistance training, and reported reduced feelings of well-being (positive affect).

4.3 Cognitive Function and Exercise

Studies have shown that physically active individuals maintain better neurocognitive function than opposing inactive individuals (64-66). However, intensity of exercise poses an added factor to acute improvements in cognitive tasks such as executive functioning, processing speed and working memory (67). Chang et al (60) suggests that exercising at high intensities or to exhaustion, impairs improvements in executive functioning tasks being assessed immediately after exercise (60). Although a recent meta-analysis conducted by Smith et al. (67) revealed that intensity improved processing speed but not executive function in aerobic, or combined exercise interventions. A combined exercise intervention, including aerobic and strength training improved processing speed to an even greater degree than aerobic alone, suggesting the hybrid intervention's superiority (67). When looking at chronic physical fitness, Folkins et al (68) found a significant improvement in cognitive measures, including increased work

efficacy correlating with increases in physical fitness measures. These findings suggests that psychological changes are closely tied to physical fitness (68). Furthermore protein supplementation has been shown to improve cognitive tasks after exercise, and even improve mood (69, 70). Walker et al. (71) suggests that whey protein, including luecine, could enhance cognitive performance during exercise or physical fatigue by staving off central fatigue. Increased concentrations of branch-chain amino acids, like luecine from the whey protein, can compete with tryptophan to enter into the central nervous system, decreasing production and accumulation of serotonin, a primary cause of decreased physical and cognitive performance (71). Walker et al. (71) also suggests that an up-regulation of the mTOR pathway per increased dietary protein intake is suspected to positively influence cognition and learning (72). However, Walker et al. (71) found cognitive tests unchanged despite proposed theoretical benefits.

5. Conclusion

A review of evidence shows that a combination of a higher protein diet in combination with anabolic forms of exercise such as resistance exercise and HIIT can be employed to increase MPS during energy balance and an energy deficit situation. Enhancing rates of MPS, through both a high protein diet and practice of anabolic exercise, during an energy deficit can result in retention of lean mass (LM), and even LM accretion, and increased rates of fat mass loss, resulting in 'high quality' weight loss. Promoting high quality weight loss is important

metabolically as well as in trying to retain, or improve, performance and function during an energy deficit. Some studies have shown FFM retention (7, 15, 16), increased performance (73), and even increased FFM following a period of energy deficit with higher dietary protein consumption and the regular performance of HIIT or RT (73). However, these studies (7, 15, 16, 73) employed only singular modalities of exercise or one particular nutritional strategy. Whether the exercise modalities and nutritional strategies act synergistically to increase MPS and retain FFM during energy deficit is unknown. A comprehensive study that is designed to incorporate all proven modalities of LM preservation during energy deficit in order to create a program that yields the 'highest quality' of weight loss has not been attempted. Furthermore utilization of validated gold-standard body composition measures to create a better understanding of changes in body composition, which have not been used previously, would advance our understanding in this area.

6. Statement of the problem

Athletes often engage in periods of sustained physical exertion, such as an off-season workout. These periods are often combined with low energy intakes which are sometimes voluntary in an effort to lose weight, or also involuntary in the situation where access to food may be limited or appetite may be suppressed. How best to sustain LM and physical function of these individuals

during these intense periods of time with low energy availability is not entirely clear and deserving of further study.

7. Rationale for the study

Athletes may voluntarily restrict their dietary energy intake to attain or maintain a low body weight or to promote leanness for aesthetic reasons or in an effort to optimize their athletic performance and competitive success. Most often, athletes chose to use a combination of dietary energy restriction as well as increased (or maintained) energy expenditure. When the desire for rapid and/or substantial changes in body weight are created via simultaneous caloric restriction and large exercise volumes this can lead to rapid changes in body composition that are sometimes undesirable since it involves the loss of substantial quantities of LM that could compromise performance. Loss of LM may lead to, or at least be associated with, decrements in strength, power, and/or ability to recover and withstand the demands of training (16, 74, 75). Depending on the degree of energy deficit, LM is lost at rapid rates when consuming a diet that contains a 'normal' protein intake of ~1.2-1.4 g/kg/d (7, 73).

Muscle protein mass gain or loss is determined by the difference between muscle protein synthesis (MPS), and muscle protein breakdown (MPB), and thus losses of lean mass, would be emphasized in conditions in which stimuli for MPS (i.e., protein and resistance exercise) were ineffectively used or absent and MPB would exceed MPS. In the case of athletes a lack of 'support' for MPS would be

seen in a marked energy deficit, poorly-timed ingestion of nutrients (i.e., temporally dissociated from exercise), along with increased intensity and duration of training, resulting in net lean mass loss.

Finding the correct macronutrient balance for athletes in an intense phase of training can be difficult, especially when energy requirements are high and the athlete may be attempting to lose weight. The most important macronutrient in promoting lean mass retention is dietary protein due to its transient but markedly potent effect in stimulating MPS (17). According to the American College of Sports Medicine protein intake for athletes should be ~1.2 for endurance athletes and between 1.7-1.8 g/kg/day for resistance training athletes (1); however, more recently pseudo-meta-analytical reviews have recommended intakes of protein much higher than this level (i.e., 2.7-3.1 g/kg/d) for resistance training athletes who are in an energy deficit (15). Resistance exercise in combination with protein can be used as an efficient simulator of MPS, increasing positive muscle NPB (18-20). Thus, when the primary goal is to retain lean mass while in an energy deficit multiple stimuli, in addition to dietary energy restriction, are essential for positive protein balance and lean mass gain. By pairing protein intake with resistance exercise an even greater MPS response is elicited than what is seen with either stimulus alone (3, 13, 20, 76). The protein dose that maximally stimulates MPS is an important consideration. Work from our laboratory has previously shown that following resistance exercise ~20g of protein, which we have recently refined down to a dose of 0.25-0.3 g/kg/meal protein dose (D.

Moore et al, manuscript in review), maximally stimulated MPS in young healthy males (13). Hence, doses in this range, or possibly greater (15), are likely beneficial during energy deficit.

It has been reported that with energy restriction lean mass gains do not occur, or are rare, since LM is most often lost during hypocaloric periods (28). However, a study from our lab in overweight and obese premenopausal women (77) as subjects challenges this notion. In this study subjects who consumed a higher protein (mostly from dairy) diet during energy restriction and exercise training not only lost fat mass, but gained lean mass, indicating that the weight lost was exclusively accounted for by fat losses (77). In athletic populations a similar result with protein consumption and practice of resistance exercise has been reported (7, 73). Although resistance exercise is clearly the most potent stimulus for muscle protein synthesis (17), there is some recent evidence that HIIT can promote lean mass preservation and even accretion (36, 40, 41).

In sum, it would be prudent, if trying to preserve LM (and promote fat mass loss to create high quality weight loss), to combine strategies such as an appropriate protein dose, protein source, protein timing as well as practicing resistance exercise and HIIT since all are important stimuli in regulating MPS and LM during energy deficit (17). To date there has not been a study that has investigated the effect of a combination of these interventions on lean and fat mass changes during a well-controlled caloric deficit with close monitoring of subjects' exercise training.

8. Aim and Hypothesis

The primary aim of this trial was to determine how dietary protein intake effected body composition and various performance and psychological variables while in a high intensity exercise program and marked energy deficit (~40% reduction in estimated energy requirements). The primary outcome of interest was body composition. It was hypothesized that during energy deficit (33±1 kcal/kgLBM/d) for 28d that consumption of a higher (2.4 g/kg/d) versus a lower (1.2 g/kg/d) protein intake would allow young men to reduce their body fat, completely preserve and possibly augment their LM, and enhance their physical function while conducting daily physical training. Secondary outcomes assessed during this intervention included psychological measures of executive function, mood isometric strength, and endurance as well as strength testing, and muscle fibre cross-sectional area analysis. While specific hypotheses concerning these variables were not made a priori, the overriding proposal was that higher protein may allow athletes to 'function better' (i.e., experience less 'stress') when consuming a higher level of dietary protein.

CHAPTER II

EFFECTS OF DIFFERING DIETARY PROTEIN INTAKES ON HYPOCALORIC DIET-INDUCED CHANGES IN BODY COMPOSITION, PHYSICAL AND MENTAL PERFORMANCE

9. Introduction

Weight loss is a desirable outcome or oftentimes an unavoidable consequence for athletes. In athletes the most commonly used means of losing weight is by dietary energy restriction, which is oftentimes combined with increased energy expenditure. For some athletes, rapid weight loss is often desired, which can be both detrimental to physical performance and mental capacity (16, 74, 75). During 'typical' diet-induced weight loss ~25% of the weight lost is lean mass (28), which has to have resulted from an imbalance in muscle protein turnover favouring net protein breakdown. Muscle net protein balance (NPB) is defined as the algebraic difference between muscle protein synthesis (MPS), and muscle protein breakdown (MPB). During energy balance following a meal MPS increases in response to the ensuing hyperaminoacidemia. The meal-induced insulinemia is primarily responsible for a small inhibition of MPB. During caloric restriction MPB tends to predominate over MPS due to a reduction in basal fasted MPS and a reduced fed-state increase in MPS (16, 42, 43). Lean mass (LM) retention during weight loss may be important for athletes to maintain performance and withstand the rigors of training; thus, strategies that stimulate MPS and inhibit MPB during energy deficit are of interest to athletes and coaches alike.

Protein consumption stimulates MPS and allows transient periods of positive NPB. When combined with performance of resistance exercise MPS is further stimulated and muscle NPB remains positive for longer allowing for LM

accretion (18). Recent evidence from Areta et al. (43) shows that higher doses of protein (30g) consumed after resistance exercise, while in a hypocaloric state, resulted in a greater stimulation of MPS. This observation (43) is in agreement with findings from Pasiakos et al. who showed that protein feedings twice the RDA were necessary to slow lean mass loss in an energy deficit (16). The suggestion from acute studies that higher protein intakes might be beneficial in stimulating MPS, and maintaining lean mass, during an energy deficit is supported by data from a recent pseudo meta-analysis, the conclusion of which was that higher protein intakes (i.e., 2.5-3 g protein/kg/d) were needed during energy deficit to preserve lean mass (5). For athletes, the American College of Sports Medicine recommends the consumption of between 1.2 g protein/kg/day for endurance athletes and 1.6-1.7 g protein/kg/day for resistance-trained athletes (1). Recent studies carried out while athletes were in energy deficit challenge this recommendation, however, and have made recommendations upwards of 2.4 g protein/kg/day (7, 16, 73). These recommendations are made based on a dietary protein-induced preservation of lean mass during energy deficit, and a result of 'higher quality' weight loss, which we define as the loss of weight comprised of the highest possible ratio of fat:lean mass.

Resistance training is also a potent stimulator of MPS (17). On its own resistance exercise increases MPS for at least 48h following an acute exercise bout (18). Resistance exercise has been shown to increase muscle cross-sectional area, meaning that over time the anabolic stimuli of the training kept

MPS rates higher than MPB resulting in net positive protein balance. Thus, the combination of higher protein intakes with resistance exercise during caloric restriction can result in a synergistic effect, creating higher ratios of fat to lean mass loss during an energy deficit (7, 28, 77).

High intensity interval training (HIIT) is an exercise stimulus that can result in rapid gains in aerobic fitness and also endurance capacity. Performing HIIT may aid in a promoting positive protein balance with some evidence to suggest that it also leads to muscle protein accretion (36, 40, 41). Given its ability to stimulate MPS, HIIT may be an advantageous modality to use to promote lean mass retention, or accretion, during weight loss.

The timing and quantity of protein intake are other factors to consider in the preservation of lean mass during an energy deficit. Our lab (4), as well as others (3, 4, 76, 78) have reported that when protein is consumed in close temporal proximity following resistance exercise, net positive protein balance is greatest (2, 30, 79); however, the net effect of protein timing with training may be relatively small. Nonetheless, pragmatic advice would be to provide protein soon after exercise to promote recovery. It may also be that during an energy deficit that the impact of protein timing would be more important that during energy balance or surfeit. The dose of this timed protein supplementation may also plays a more important role in optimally preserving skeletal muscle mass during an energy deficit. A dose of ~20-25g of protein (refined recently to a dose of 0.25-0.3 g protein/kg/meal) optimally stimulates muscle protein synthesis in young males

following resistance exercise (13, 80). Thus, it would seem prudent to recommend early post-exercise protein ingestion of a sufficient quantity of protein to aid in lean mass retention while in an energy deficit.

While recent studies have shown lean mass sparing effects with resistance exercise (7, 73), a study that has employed effective protein timing, HIIT, provision of high quality protein supplement during a controlled dietary period, has not been performed. We aimed to determine whether a combination of all of the aforementioned strategies would completely attenuate loss of LM, or potentially promote LM accretion. Thus the purpose of this study was to evaluate the effectiveness of all modalities of protein anabolism combined on body composition changes when paired with either a high protein (PRO) or a normal protein (CON) diet in a controlled manner over 28 days of severe energy deficit. We hypothesized that the PRO group would retain all, if not increase, their LM. Moreover we hypothesized that strength and muscle fibre cross sectional area would be increased to a greater degree in the PRO group.

10. Methods

10.1 Subject Recruitment

Forty overweight (BMI >25 kg/m^2), recreationally active young men (19-30 years) were recruited from the local Hamilton community and McMaster University. Subjects were screened to exclude smokers and people with health conditions that might affect their response to the protocol. Subjects had to have a maximal

aerobic capacity ($VO_{2\ peak}$) > 35ml/kg/min and a body fat (BF) percentage >15%. The study was approved by the Hamilton Health Sciences Medical Research Ethics Board and was in full compliance with the most up-to-date Canadian tri-council statement on the use of human subjects in research (http://www.pre.ethics.gc.ca/pdf/eng/tcps2/TCPS_2_FINAL_Web.pdf). All subjects gave their informed written consent prior to commencement of the study. Upon obtaining consent, subjects were randomized to one of two groups: control (CON): energy restricted diet (33 kcal/kgLBM/d; 15% protein, 50% carbohydrates, 35% fat) with 1.2g/kg protein/d; or higher protein (PRO): energy restricted diet (33 kcal/kgLBM/d; 35% protein, 50% carbohydrates, 15% fat) with 2.4 g/kg protein/d. The subjects' descriptive characteristics are given in Table 1.

Table 1. Subject anthropometric characteristics.

Group	Age(y)	Weight(kg)	Height (m)	BMI(kg/m^2)
PRO (n=20)	23±2	100.1±12.8	1.84±0.06	29.7±3.9
CON (n=20)	23±3	96.0±14.6	1.80±0.09	29.6±2.7

Values are means ± SD.

10.2 Experimental Protocol

Pre-testing was conducted over six days and is detailed below. Subjects reported to the laboratory and underwent familiarization for all testing, 1 repetition maximum (1-RM) chest and leg press, isometric maximal voluntary contraction of

the knee extensors using an Biodex dynamometer (3.0, Shirley, New York) and training (leg press, bench press, shoulder press, lateral pull down, bicep curls, tricep extension, leg extension and leg curl). On a separate day, subjects reported to the lab and underwent a progressive maximal aerobic capacity test (VO_{2peak}) on a cycle ergometer, using a 30-Watt Ramp protocol. On a subsequent day, subjects reported to the lab and underwent a maximal voluntary contraction (MVC) of the knee extensors (isometric knee extension at 90 degrees) using the Biodex dynamometer. Subjects then underwent a Wingate bike test to determine peak anaerobic power and a sit up (number of supervised 'good form' sit ups completed in 1 minute) (81) and push-up test (number of supervised 'good form' pushups to voluntary exhaustion) (82). On the following day, subjects reported to the laboratory and underwent 1-RM testing for chest press and leg press using established protocols to ensure consistency (82). On the sixth day of pre-testing, subjects reported to the laboratory and underwent psychological testing. Subjects filled out two baseline questionnaires, the Positive and Negative Affect Scale (PANAS, Shortened Version) (83) to determine the subjects' emotional state and Profile of Mood States (POMS, Shortened Version) (84) to determine their mood state. Subjects then underwent four psychological tests in the same order for all subjects following a 5 min low-intensity walk. Isometric maximum voluntary contraction (MVC) was determined using a digital handgrip dynamometer, response rate and error frequency was found using the modified Stroop test (85),

executive control was looked at using the operation span test (OSPAN) (86, 87), and lastly reaction time was determined using the vigilance task (88).

Subjects were then provided with a 3 day weight maintenance diet based on the Harris-Benedict equation (89), using an appropriate activity factor for the days leading up to their muscle biopsy (see Nutrition below for details). On the third day of the maintenance diet subjects reported to the laboratory after a 10h overnight fast and had a muscle biopsy taken from the vastus lateralis, as described previously (22, 33, 90), as well as a blood sample from an antecubital vein. The muscle biopsy sample was processed for further analysis. One piece (~30mg) of the sample was mounted in optimal cutting temperature (OCT; Tissue Tek, Sakura) medium in liquid nitrogen-cooled isopentane and then subsequently snap frozen and stored at -86ºC until analysis.

Heparinized blood was immediately placed on ice whereas serum samples were allowed to clot at room temperature for ~15min. Blood samples were then spun at 4000 rpm for 10min at 4C and plasma and serum fractions were stored at -20C until subsequent analysis. Subjects also underwent BF determination by BodPod (Cosmed, California, USA), a whole-body scan by dual-energy x-ray absorptiometry (DXA, QDR-4500A; Hologic), and measurement of total body water by bio-electrical impedance (BIA, Maltron; Raleigh, UK) scans for determination of body composition.

10.3 Nutrition

Subjects received all of the food they were to consume throughout the study by use of pre-packaged foods, frozen ready-to-eat meals, and drinks. Every time subjects received their food they were given instructions on when to consume each meal. Compliance with the nutritional intervention was assessed by frequent (daily) contact with subjects, food checklists, and daily weight monitoring. Upon completion of the 4-week dietary and physical activity intervention subjects repeated all testing (except familiarization) in the same manner and order.

Prior to entering the energy restriction phase each subject was given a three-day maintenance diet to lead up to biopsy day both prior to and following the intervention. Maintenance diets were created using body composition measures from the pre Bod Pod measurement. The Harris Benedict equation with correction for activity factors was used to estimate energy intake. Macronutrient intake during the maintenance phase was ~15-18% protein, 55-60% carbohydrate, 20-25% fat. All subjects were on the same diet leading into pre and post blood samples, muscle biopsies, strength, and psychological measures.

Each subject was given a personalized energy-restricted nutrition plan that included all meals, protein drinks, snacks, and instructions on when to eat them for the entire 28d intervention. Subjects were placed on a three-day rotating nutrition plan and all meals were pre-packaged, frozen meals from Copper

County Foods (Hamilton, Ontario). Both groups received a whey-containing shake that formed the primary nutritional intervention that altered their dietary macronutrient content. Subjects consumed one of these drinks immediately post workout at the lab under supervision. The PRO group had an intake of 2.4g/kg/d of protein while the CON group had 1.2g/kg/d. The total dietary energy intake equated to 33kcals/kg fat-free mass (FFM)/d. The estimate of FFM measurement to create the diet was obtained from pre-intervention measure of BF obtained the Bod Pod. The percentage goal of each macronutrient was as follows: PRO – 35% protein, 50% carbohydrates (CHO), 15% fat; and CON – 15% protein, 50% carbohydrates, 35% fat. Fat content of the diets was altered in the intervention groups to avoid performance advantages due to differences in total CHO intake. Macronutrient breakdown and energy restriction for both groups can be found in Table 2.

Table 2: Composition of energy-restricted diets.

Group	Kcals	Protein(g)	CHO(g)	Fat(g)
PRO (n=20)	2560 ± 282	245 ± 31	311 ± 35	38 ± 6
(g/kg)		2.4 ± 0.1	3.1 ± 0.3	0.4 ± 0.1
CON (n=20)	2374 ± 304	116 ± 19	286 ± 35	86 ± 13
(g/kg)		1.2 ± 0.1	3.0 ± 0.2	0.9 ± 0.1

Values are means±SD.

Each group received 3-4 protein-containing drinks/d depending on their body weight. These drinks were responsible for the majority of (~58%) blinding of the subjects to what group they were in. Specific high protein or low protein meals were ordered to have bolus' of protein spread out throughout the day, not just having protein alteration coming from the macronutrient altering shakes. Fat content was altered significantly by provision of either whole milk (3.25% milk fat) in the CON group, or skimmed (0% milk fat) milk in the PRO group. Both drinks were flavoured with chocolate powder (Nestle Nesquik) resulting in no distinct taste difference between groups. Drinks were also altered in protein content using Agropur IsoChill 9010 Instantized Whey Protein Isolate. Maltodextrin was added to each of the drinks in order to keep protein:CHO ratios similar between groups. Wanted to control for carbohydrates, focusing on the effects of protein supplementation throughout the trial, thus an altered post workout shake for the PRO group was created to retain similarities between protein:CHO ratios using the Maltodextrin. Drink macronutrient composition is shown in Table 3.

Table 3: Composition of supplemental drinks.

Group	Kcals	Protein(g)	CHO(g)	Fat(g)
PRO (N=20)	372 ± 35	49 ± 6	48 ± 7	2 ± 0
Post	532 ± 35	49 ± 6	88 ± 6	2 ± 0
CON (N=20)	330 ± 56	15 ± 4	41± 6	12 ± 3

PRO, 2.4 g/kg/d protein; CON, 1.2 g/kg/d protein. PRO had an extra 40g of maltodextrin added to their post-workout (Post) drink to retain similar protein:CHO ratios throughout the trial. CON same drinks throughout. Shakes given at lab and consumed before leaving. Values are means±SD.

10.4 Exercise Training

Subjects reported to the laboratory for six training sessions each week. Training sessions followed a rotating series of exercises designed to bring about increases in aerobic fitness and strength. Thus, two d/week subjects performed full-body resistance training (Tuesday and Thursday): 2 circuits/day, 5 exercises/circuit (circuit 1: bench press, knee extension, tricep extension, hamstring curls, sit ups; circuit 2 – lateral pulldown, shoulder press, bicep curls, leg press, bicycle crunch), 3 sets/circuit, 10 repetitions/set for sets 1 and 2 and to failure for set 3. Subjects had a 1-minute rest between sets within a circuit and a 3-minute rest between the end of the first circuit and the start of the second circuit.

1) 2 days/week high-intensity interval training (HIIT, Monday and Wednesday): Subjects underwent 2 different HIIT regimes as follows. 1) 4-8 30 s Wingate tests (load determined based on 0.075% of body weight) with 4 min rest between bouts and 2) 10 bouts of 1 min at 90% peak wattage (as determined from VO_{2peak}) with 1 min rest between bouts.
2) Time trial (Friday): Time to complete a 250 kJ ride on a cycle ergometer. Subjects controlled their wattage throughout the time trial and were instructed to complete the time trial as fast as they could. Subjects were encouraged throughout the time trial.
3) 1 day/week plyometrics (Saturday) shown in Table 4.

Table 4: Plyometric Exercises

Circuit	Exercise	Time	Rest	# of Sets
1st	Burpees Fast Jacks Shuffle Toe Touches	1 min	1 min	3
Rest	-	-	2 min	-
2nd	Jumping Lunges Crab Kicks Planks	1 min	1 min	3
Rest	-	-	2 min	-
3rd	Jump Squats Push-ups Plate Twists	1 min	1 min	3

Subjects were instructed to consume a second protein drink 1h prior to sleep each evening. Subjects were also given a pedometer and were instructed to accumulate at least 10000 steps/d throughout the trial. Subjects step counts were monitored on a daily basis and averaged 11915 ± 2492 steps/day. At the end of each week of training subjects had a fasted blood sample taken and also entered disposition of moods for the week with the PANAS and Profile of Mood States (POMS) tests.

10.5 Body Composition

Three different body composition modalities were performed in order to calculate a 4-compartment (4C) body composition model as described previously (91). This model differentiates and corrects assumptions about bodily voids when calculating total body volume (TBV). The 4C model is considered the gold standard in body composition and uses mass, TBV (Bod Pod), total body water (BIA), and bone mineral content (BMC; DXA) to calculate percentage body fat, and lean body mass. The equation is as follows (91):

$$[\%\ \text{Fat} = 100 \times (2.747/(\text{Mass}/\text{TBV}) - 0.714 \times \text{TBW}/\text{Mass} + 1.146 \times \text{BMC}/\text{Mass} - 2.050)]$$

All body composition measures were taken in the fasted state and at the same time of day. In order to minimize between-subject variability subjects were provided with a 3-day maintenance diet, as detailed above, and instructed to abstain from physical activity for 48 hours prior to the biopsy and body composition testing day.

Whole-body DXA scans (software version 12.31) were carried out prior to and following the intervention to determine BMC for the four-compartment model. Participants were instructed to wear light clothing with no metal buttons or zippers. Participants laid flat on the DXA table and a whole-body scan was commenced. All measurements were taken after an overnight fast between 0730 and 0930.

Measurements of TBV were acquired using the Bod Pod software (Life Measurement Instruments Inc., California, USA). Participants reported to the laboratory immediately after their DXA scan. The BodPod was calibrated every morning and before each participant. Placement of a hollow cylinder with a known mass and volume was placed into the Bod Pod for calibration purposes. Participants wore a tight-fitting swim cap, and were instructed to wear tight-fitting or spandex-like shorts in order to reduce the effect of isothermal air on the measurement. The participants were then sealed in the Bod Pod, and two measurements of body volume were conducted in sequential order. If there was too great of a discrepancy between the first and second measurement (inter-assessment difference >5%), then a third measurement was taken.

Total body water (TBW), was measured using the Maltron Bio-Scan 920-II multi-frequency bio-impedance analyzer. Participants completed this measure immediately following the Bod Pod measurement and were instructed to lie down on a medical table and relax for 5 minutes to allow for water equilibration. Four

Kendall 5400 diagnostic electrode pads were placed on the right side of the body. Data was collected and analyzed using Maltron BioScan 920 Software.

10.6 Strength

Isometric knee extension torque was measured using the Biodex System 3 version 3.29 & 3.30 (Medical Systems). Participants were familiarized with the instrument and protocol before testing on a separate day. Seat adjustments were made and recorded so pre and post tests were comparable. Participants were strapped into the seat and instructed to cross their arms in front of their body, and exert a maximal isometric knee extension contraction with their dominant leg for 5 seconds, followed by a 30 second rest for three attempts. Maximal voluntary contraction torque during any of the three attempts (MVC) was recorded and analyzed.

Single lift isotonic strength (1-RM) testing was conducted in the exercise-testing lab using free weights and the ACSM testing protocol for muscular strength. The chest press was conducted on a Badger Magnum bench (Hammer Strength, Lake Forest, IL), and the Leg Press was conducted on a Maxam Strength Press (Maxam, Hamilton, Ontario), both using standard weight plates. Participants were familiarized on a separate day with both exercises, using a warm-up and finding a weight the subject found comfortable to do ~10-15 repetitions. Using these numbers an estimated 1-RM was determined in order to gauge load for 1-RM testing day (82). On testing day, subjects were instructed to ride a cycle ergometer for 10 minutes and to warm-up on the bench/leg press

with a set of 8-10 repetitions of a weight found light to the participant. An initial weight was then selected within the subject's perceived capacity (~50-70% of estimated 1-RM). Resistance was progressively increased until the subject could not complete 1 repetition at the same speed of movement and range of motion. 1-RM was determined within 4 attempts with rest periods of 3-5 minutes between attempts. The final weight lifted successfully was recorded as the absolute 1-RM.

Push-up tests were conducted following ACSM muscular endurance protocol (82). The protocol began with the subject in the standard down position (hands pointing forward and under the shoulder, back straight, head up, using the toes as the pivot point). The subject then had to raise his body by straightening their elbows and return to the 'down' position until chin touches the mat or ground, not the stomach. The maximal number of push-ups performed consecutively without rest was counted as the subjects' score. The test was halted when the subject was unable to maintain the appropriate technique or voluntary failure occurred.

Sit-up tests were conducted following the Canadian Forces Military protocol (81). Subjects were instructed to lie flat on their backs with knees bent at 90 degrees. Feet were flat on the floor, shoulder width apart, with a personal trainer (counter) standing directly on them. Arms were crossed across subject's chest to avoid momentum swings. To complete one sit-up subjects must sit-up and bring elbows to the top of one's knees, and then lower back down to start position. Participants performed as many sit-ups as possible in a 60s time frame.

Maximal aerobic capacity ($VO_{2\ peak}$) testing was conducted pre- and post-intervention using the Moxus Modular VO2 System, with Oxygen Analyzer S-3A/1. The instrument was calibrated every morning. On the test day subjects were instructed to warm-up for 10 minutes on a cycle ergometer beforehand. They then completed a 30-Watt Ramp protocol using a LODE Excalibur (8V Medical Technology, Groningen, Netherlands) cycle ergometer. Participants were encouraged throughout the test. The test started by the subject cycling and reaching a workload of 30W, and increasing 1W/s. The subject was instructed to keep a cadence of 60-70rpm and terminated once the rpm could longer be retained.

Wingate testing was conducted on the Wingate Velotron Racemate (Seattle, WA). Subjects were instructed to warm-up on a cycle ergometer upon arrival. They then followed a Wingate anaerobic test (WAT). Using Velotron Wingate Software v1.0, load was set to 0.075kg/kgBW Subjects began pedaling at which time the load was imposed and the subjects were told to pedal 'all out' for a period of 30s, with encouragement from the personal trainer. This test was completed pre and post intervention and was analyzed for peak power, mean power, and fatigue index.

10.7 Immunohistochemistry

Muscle samples mounted in OCT were analyzed for muscle fibre type and cross-sectional area using immunofluorescent microsopy. Muscle cross sections (7 µm) were cut from OCT-embedded tissue using a Cryostat (Thermo Scientific Microm

HM 550) onto slides, air dried and then stored at -80℃. Briefly, tissue sections were fixed in 4% paraformaldehyde for 10 min, washed 3x for 5min in phosphate buffered saline with 2% tween (PBST), blocked for 60min at room temperature in PBS containing 2% BSA, 5% FBS, 0.2% Triton X-100, 0.1% sodium azide, and 2% goat serum. Slides were then stained with antibodies against MHCI; slow isoform; neat; DSHB); myosin heavy chain type II (MHCII; fast isoform; 1:1000; ab91506, Abcam, Cambridge, MA, USA); neonatal myosin heavy chain (nMHC 1:10; VP-M666; Vector Laboratories, Burlingame, CA, USA); laminin (1:1000; ab11575, Abcam). Secondary antibodies used were MyoD (biotinylated secondary antibody, 1:200; Vector Canada, Burlington, ON, Canada, and streptavidin-594 fluorochrome, 1:500; Invitrogen, Molecular Probes); A4.951 (Alexa Fluor 488, 1:500); MHCII (Alexa Fluor 647, 1:500); nMHC (Alexa Fluor 488, 1:500); and laminin (Alexa Fluor 647, 1:500). Staining specificity was confirmed using appropriate negative controls. Samples were viewed with the Nikon Eclipse Ti microscope at x20 view and captured with a Photometrics CoolSNAP HQ2 fluorescent camera (Nikon Instruments, Melville, NY, USA), and images were captured and analyzed using the Nikon NIS Elements AR 3.2 64 bit software (Nikon Instruments). Fiber CSA was assessed with immunofluorescence staining for type I (MHCI) and type II (MHCII) fibers, and fibers expressing both MHCI and MHCII were identified as hybrid fibers.

10.8 Psychological Measures

Subjects had a separate pre-testing day for psychological measures. Upon arrival, subjects walked at low intensity pace with the personal trainer to the testing room as a means of light exercise to clear the mind. Subjects completed two paper and pencil questionnaires, the Positive and Negative Affect Scale (PANAS) to determine the subjects' emotional state and Profile of Mood States (POMS) to determine their mood state. These questionnaires were filled out again at the end of every week before exercise. Subjects then underwent four psychological tests in the same order for all subjects, Isometric voluntary contraction (MVC) was found using the hand-grip, response rate and error frequency was found using the Stroop test, working memory was looked at using the operation span test (OSPAN), and lastly reaction time was determined using the vigilance task. These tests were conducted 2 times pre, and post-intervention in the same manner.

Handgrip exercises were performed using an isometric handgrip dynamometer (model MLT003/D: AD Instruments) with graphic computer interface (PowerLab 4/25T; AD Instruments, CO). Subjects were seated and instructed to keep the elbow of their dominant hand on the table and the target force was determined. Two 4-second MVC's were performed separated by 2min rest intervals. A maximum force generation value was calculated using the peak force during a 1s epoch at the peak of the greater MVC. Peak force was then used to calculate the submaximal (50% of peak) value at which the isometric

handgrip task was to be completed. Participants were asked to hold handgrip force at 50% of the MVC force and to maintain this for as long as possible. The monitor provided output as a real-time graphed line that indicated the actual force the participant was exerting. When the force tracing fell below the 50% MVC threshold, participants received a verbal cue of encouragement. When the participant fell below the threshold for longer than 2s the test was terminated. Participants had no knowledge of time, or the magnitude that the threshold was set at. Squeeze duration (time elapsed @ 50% peak MVC) and peak MVC were further analyzed.

A modified Stroop task (85) was conducted using five 8.5 x 14-inch papers, containing two columns with 26 words a sheet. Participants were instructed to start with the left column and read the words in a downward fashion before moving to the next column and then the next page for five minutes. Once the subjects had finished all pages, if there was time remaining, they would go back to page one and follow the same instructions. The modified Stroop task (85) requires participants to read the words aloud and tests two rules looking at cognitive inhibition. The words were colours but the ink-colour of the words were mismatched. For example, when the word 'blue' was printed in 'yellow', participants were instructed to say aloud 'yellow'. The second rule was an inclusion of a superordinate rule that required the participant to now override the original instructions when they encountered words printed in 'red' ink. Now participants were instructed to read aloud the printed word in this case. For

example if 'blue' was printed in 'red' ink, the participant was supposed to say 'blue'. This task involves more working memory and increasing executive functional demands. The total number of words read over the 5 minutes was recorded and then used to calculate average response speed by dividing by 300 seconds. The number of errors was also recorded and error frequency calculated by dividing by the number of words completed.

Participants took part in an operation span task (86, 87). Participants were seated in front of a computer screen, which successively presented them with simple math problems paired with a word. The participant was asked to read the equation aloud and state if the provided answer was correct or not and then say the word aloud immediately after. Participants were informed that they could take up to 20 seconds to correctly verify each equation, and they were instructed to start reading each equation aloud as soon as it appeared on the screen. Additionally, subjects read the word aloud immediately after verifying the equation aloud. Once the participant read the word aloud, the researcher would immediately press a key on a computer keyboard to continue on to either the next equation-word pair or to the recall cue (three question marks displayed in the center of the computer screen). For each set, the participants saw 2 – 5 equation-word pairs, and these sets were shown to all participants in the same initially randomized order (consisting of three trials at each set size, as well as three 2-item sets used as practice). At presentation of the recall cue, participants tried to write down all of the words from the current set in the order they were presented.

Also, as recommended by Engle et al., (86) to ensure that participants are not paying less attention to problem solving in order to remember more words, it was required that all participants achieved at least 85% accuracy on the math problems for their results to be included in the study. The task was scored by calculating the percentage of words in each set that were recalled in the proper serial position.

Participants completed the Sustained Attention Response Task (SART) (88). The SART is a continuous go/no-go paradigm in which participants were asked to respond to a stimulus presented on a computer monitor only on "go" trials by pressing the space bar as quickly as possible and withhold their response on "no go" trials. The "no go" trials occur infrequently (10% of the time), which promotes the establishment of a dominant pattern of pressing the space bar in response to stimulus presentation. The non-target stimuli were numbers 0 – 2 and 4 – 9 ("go" response) and the target stimulus was the number 3 ("no-go" response). All stimuli were presented in a black font on a white background. To perform optimally, participants must inhibit their urge to press the spacebar on "no-go" trials. The two measures that were analyzed were reaction times for each "go" trial, and the error rates (failure to inhibit response) on "no-go" trials.

Subjects completed the Shacham shortened version (37 item) Profile of Mood States (84). This test was used to assess transient distinct mood states at the end of every week. Adjectives (37 in total) were to be rated by each subject on a 5-point scale from 'not at all' (1 point) to 'extremely' (5 points). Adjectives

were then divided into six factor-based subscales; Tension-Anxiety, Depression-Dejection, Anger-Hostility, Fatigue-Inertia, Vigor-Activity, and Confusion-Bewilderment. Total mood disturbance was also calculated by subtracting the score of Vigor-Activity from the sum of the other five subscales. Subjects were instructed to fill out the questionnaire at the lab before exercise, on the last day of training for the week (Saturday) basing their responses on their experiences in the past week.

Subjects completed a Positive and Negative Affect Schedule (PANAS) at the end of every week of the intervention as well. This was a 20-item PANAS (83) questionnaire where subjects rated adjectives based on a 5-point scale ranging, from 'very slightly or not at all' (1 point) to 'extremely' (5 points). Words were then scored on two separate subscales measuring positive and negative affect. Subjects were instructed to fill out the questionnaire at the lab before exercise on the last day of training for the week (Saturday) basing their responses on their experiences in the past week. Positive and negative words were added up for a total each week and means were compared throughout the intervention.

10.9 Statistical Analysis

Data were analyzed using a 2-way, repeated measures analysis of variance (ANOVA) with diet (between) and time (within) being the experimental variables. Significant interaction effects were analyzed using a Tukey post-hoc test to determine the location of pair wise differences when appropriate within (time) and/or between (diet). All analyses were conducted at the 5% level. Between

group percent change comparisons were analyzed using an unpaired Student's t-test. Significance was set to $p<0.05$. Analyses were performed using SPSS (version 20.0.0). All data are presented as means ±SD.

11. RESULTS

11.1 Four-Compartment Model

Both PRO and CON lost fat mass following 28 days of energy restriction and high intensity training ($P<0.05$; Figure 7); however, fat mass losses were shown greater using a paired t-test in the PRO (4.6±1.6kg) group as compared with CON (3.5±1.4kg; $p<0.05$). Both PRO and CON retained a significant amount of lean mass ($p<0.05$) (Figure 8); however greater lean mass gains were found using a paired t-test in the PRO (1.04±1.20kg) group in comparison to the CON (0.07±1.71kg) ($p<0.045$).

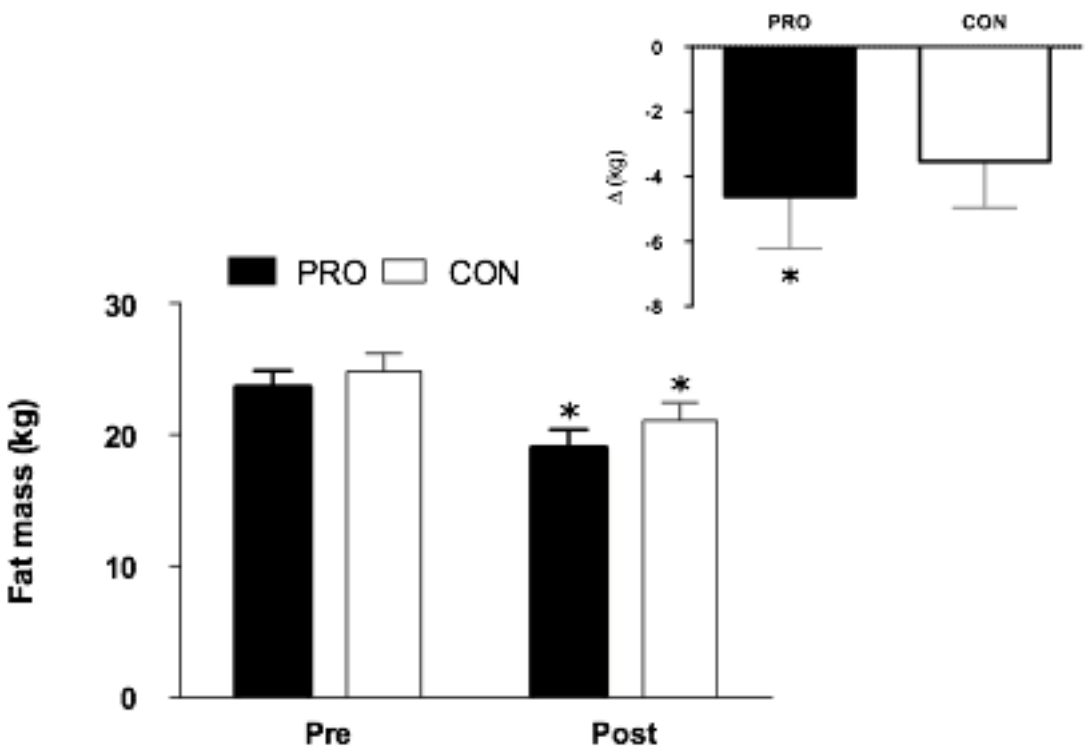

Figure 7. Fat mass derived from the four-compartment model in the PRO and CON groups Pre- and Post-intervention. * Significantly different from Pre. Inset. Change in fat mass derived from 4-compartment model. * Significant difference between groups. Values are means ± SD.

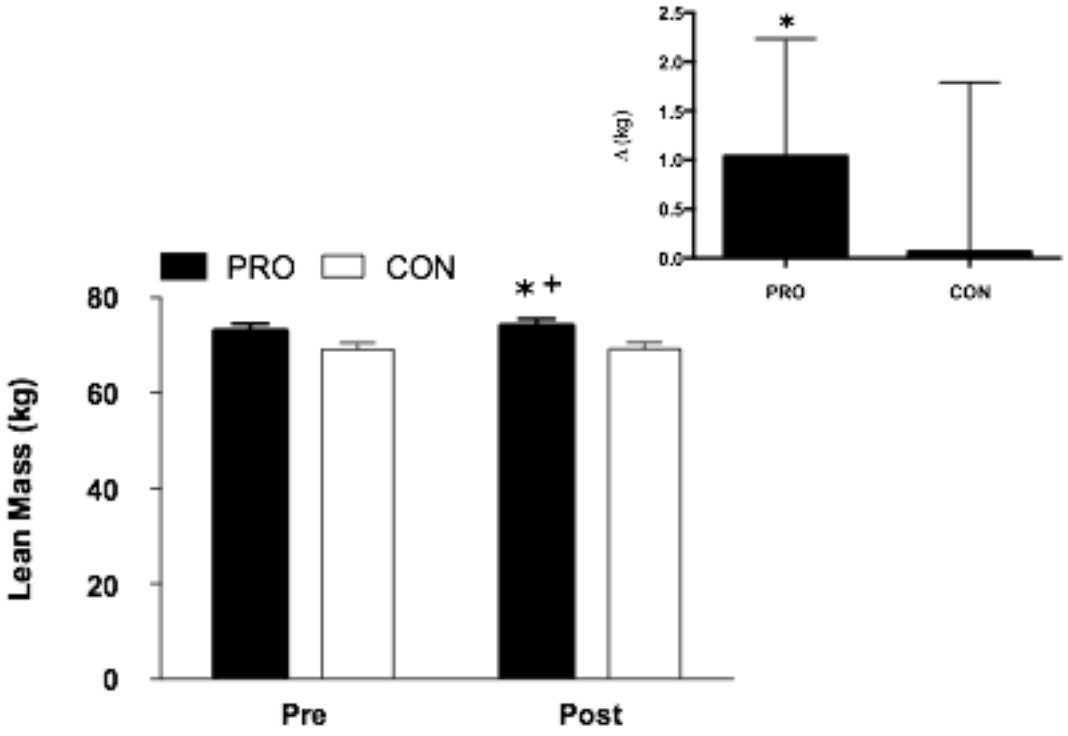

Figure 8. Lean mass derived from the four-compartment model in the PRO and CON groups Pre- and Post-intervention. * Significantly different from Pre. + Significantly different from CON within the same time. Inset. Change in fat mass derived from 4-compartment model. * Significant difference between groups. Values are means ± SD.

11.2 Strength and Performance

With the exception of isometric knee extension, strength increased for all measures in both groups ($P<0.001$). Results from all strength measures and the exact P values can be found in Table 5. There were no differences in the strength response to training between groups.

Table 5: Strength and other Performance Results

Group	Test	Pre	Post	Diff	Time (p)	TimexGroup (p)
PRO	**Leg Press**	377±60	439±78	62±42	0.00	0.75
CON	(kg)	337±70	397±71	60±35		
PRO	**Bench Press**	107±29	112±25	5±7	0.00	0.11
CON	(kg)	89±16	97±19	8±8		
PRO	**Push Up**	29±12	39±10	10±6	0.00	0.19
CON		24±10	31±12	7±4		
PRO	**Sit Up**	36±9	47±8	11±5	0.00	0.24
CON		33±12	40±13	7±5		
PRO	**Peak Power**	1148±130	1277±133	129±114	0.00	0.72
CON	(W)	1095±249	1237±205	142±129		
PRO	**Mean Power**	768±76	805±76	37±58	0.01	0.94
CON	(W)	707±83	743±98	36±70		
PRO	**Total Work**	23029±2295	24104±2271	1075±1806	0.01	0.78
CON	(J)	20883±2696	22237±2986	1354±2121		
PRO	**VO_2 Max**	41.1±5.6	47.2±7.8	6.1±4.8	0.00	0.15
CON	ml/kg/min	40.5±4.9	44.6±6.4	4.1±3.2		
PRO	**Biodex** (N.m)	329±59	336±67	7±33	0.65	0.27
CON		294±52	291±53	-3±21		

PRO, 2.4 g/kg/d protein; CON, 1.2 g/kg/d protein. Values are means ± SD.

Weekly time trial results are detailed in Table 6. There was a main effect for time and post hoc analysis indicated that time trial performance improved in both groups over the course of the intervention ($p<0.005$).

Table 6: Time Trial Results (min:sec).

Group	Wk 1	Wk 2	Wk 3	Wk 4	Wk4-Wk1
PRO	19:09±3:54	17:03±3:16	16:33±3:20	15:37±2:53	3:32±1:59
CON	21:13±3:43	19:03±2:44	18:03±2:44	17:09±2:22	4:10±2:55

PRO, 2.4 g/kg/d protein; CON, 1.2 g/kg/d protein. Significant main effect for time. Values are means ± SD.

11.3 Immunohistochemistry

There was an increase in Type 2 muscle fibre cross-sectional area (CSA) in both the PRO (844±2149um^2) and CON (519±2090um^2) groups ($p<0.05$, Figure 3). There was an overall increase in CSA (increase in Type 1, Type 2 and hybrid fibre CSA) ($p<0.05$) in both groups (Figure 4). There was no effect of the intervention on muscle fibre type proportions, Type 1 or hybrid CSA.

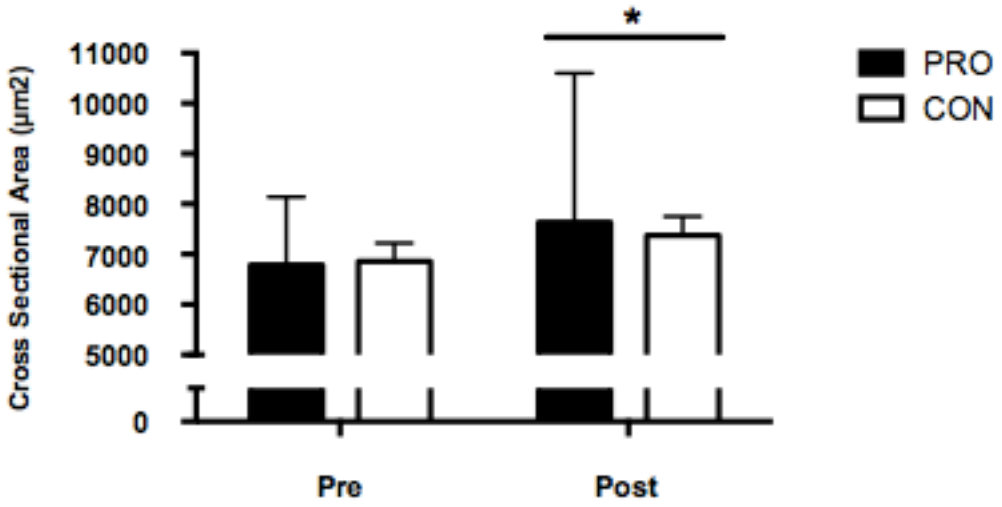

Figure 9. Type 2 muscle fibre cross-sectional area (um^2) prior to and following 28 days of energy restriction and high intensity training in the CON and PRO groups. *significant main effect for time. Values are means ± SD.

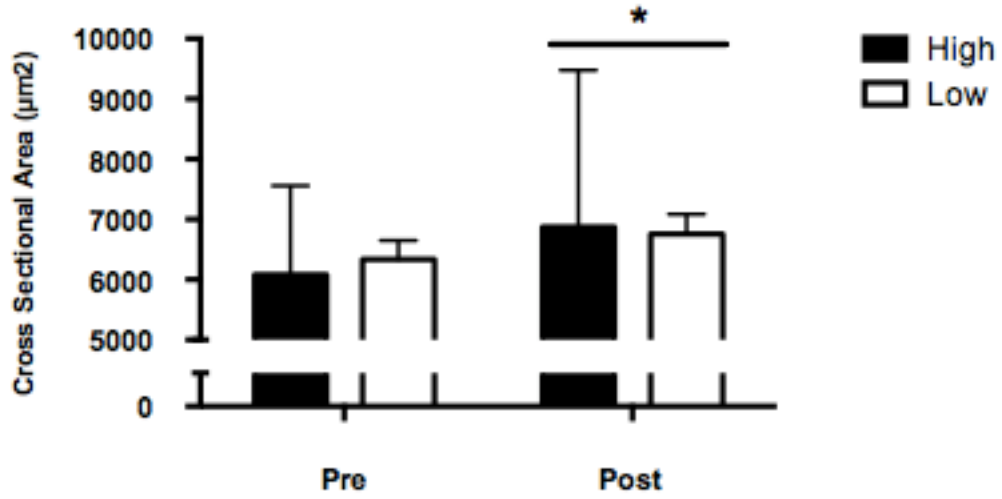

Figure 10. Total muscle fibre cross-sectional area (um^2) prior to and following 28 days of energy restriction and high intensity training in the PRO and CON groups. *significant main effect for time. Values are means ± SD.

11.4 Psychological Measures

Weekly mood questionnaire results are found in Tables 7 & 8. The Profile of Mood States questionnaire showed significant main effects for time in confusion, tension, and fatigue at specific time points using post hoc analysis. Confusion showed a significant decrease between pre-wk3, pre-wk4, wk1-wk3 & wk1-wk4 ($p<0.05$) in both groups. Tension showed significant decreases between time points pre-wk2, pre-wk3, wk1-wk2, wk1-wk3, & wk1-wk4 ($p<0.05$). Fatigue showed significant increases in reporting for pre-wk1, pre-wk2, pre-wk3, and then decreases for wk1-wk2, wk1-wk3 & wk1-wk4 ($p<0.05$).

Table 7: Profile of Mood States Results

POMS	Pre	Wk1	Wk2	Wk3	Post
Depression					
PRO	$0.29\pm0.57_{a}$	$0.43\pm0.67_{a}$	$0.27\pm0.29_{a}$	$0.26\pm0.30_{a}$	$0.29\pm0.45_{a}$
CON	$0.33\pm0.65_{a}$	$0.39\pm0.64_{a}$	$0.29\pm0.53_{a}$	$0.32\pm0.70_{a}$	$0.52\pm1.01_{a}$
Vigor					
PRO	$2.32\pm0.57_{a}$	$2.19\pm0.75_{a}$	$2.13\pm0.75_{a}$	$2.11\pm0.80_{a}$	$2.22\pm0.87_{a}$
CON	$1.72\pm0.50_{a}$	$1.97\pm0.81_{a}$	$2.01\pm0.91_{a}$	$1.94\pm0.81_{a}$	$2.04\pm0.85_{a}$
Confusion					
PRO	$0.78\pm0.63_{a}$	$0.73\pm0.65_{a}$	$0.58\pm0.51_{b}$	$0.47\pm0.58_{c}$	$0.47\pm0.68_{c}$
CON	$0.79\pm0.64_{a}$	$0.82\pm0.75_{a}$	$0.73\pm0.67_{b}$	$0.60\pm0.84_{c}$	$0.61\pm0.76_{c}$
Tension					
PRO	$0.80\pm0.58_{a}$	$0.78\pm0.60_{a}$	$0.55\pm0.50_{b}$	$0.53\pm0.50_{ac}$	$0.49\pm0.56_{c}$
CON	$0.89\pm0.77_{a}$	$0.99\pm0.75_{a}$	$0.69\pm0.65_{b}$	$0.73\pm0.74_{ac}$	$0.75\pm0.91_{c}$
Anger					
PRO	$0.34\pm0.52_{a}$	$0.53\pm0.57_{a}$	$0.39\pm0.47_{a}$	$0.24\pm0.38_{a}$	$0.21\pm0.31_{a}$
CON	$0.45\pm0.62_{a}$	$0.55\pm0.62_{a}$	$0.51\pm0.54_{a}$	$0.54\pm0.71_{a}$	$0.47\pm0.85_{a}$
Fatigue					
PRO	$0.96\pm0.79_{a}$	$1.75\pm0.84_{b}$	$1.40\pm0.63_{c}$	$1.43\pm0.90_{cd}$	$1.07\pm0.95_{acd}$
CON	$1.06\pm0.81_{a}$	$1.82\pm0.90_{b}$	$1.60\pm0.68_{c}$	$1.31\pm0.80_{cd}$	$1.28\pm1.02_{acd}$

PRO, 2.4 g/kg/d protein; CON, 1.2 g/kg/d protein. Significant main effect for time. Values in the same row that do not share common subscript differ ($p<0.05$). Values are means ± SD.

Positive and Negative Affect Schedule (PANAS) showed significant main effects for time at specific time points using post hoc analysis ($p<0.05$). Positive

affect significantly decreased with time for pre-wk2, & pre-wk3, while significantly increasing wk3-wk4 ($p<0.05$).

Table 8: Positive and Negative Affect Scale

PANAS	Pre	Wk1	Wk2	Wk3	Post
PA					
Pro (n=20)	$35.90\pm6.79_{abc}$	$33.50\pm7.92_{abc}$	$32.95\pm7.76_{bc}$	$32.20\pm8.73_{bc}$	$35.55\pm10.4_{a}$
Con (n=17)	$30.29\pm8.25_{abc}$	$30.71\pm8.85_{abc}$	$29.41\pm7.78_{bc}$	$28.41\pm8.40_{bc}$	$31.65\pm8.18_{a}$
NA	13.60±3.46	12.95±2.72	11.85±2.58	11.70±2.27	11.45±2.35
	13.65±4.78	13.65±5.78	13.06±4.91	13.29±5.49	13.76±6.62

PANAS questionnaire weekly results. Significant main effect for values that do not share a common subscript ($p<0.05$).. PA; Positive Affect, NA; Negative Affect. Values are means ± SD.

Cognitive functioning pre/post results are found in Tables 9-12. The handgrip test showed a significant decrease for squeeze duration in both groups ($p<0.05$) with an increase in rate of perceived exertion (RPE) ($p<0.05$). There was no significance found in the vigilance task or operation span pre/post. Results of the Stroop test found significant increases in word reading speed ($p<0.05$), and decreases in the number of words wrong ($p<0.05$), relative error frequency(%) ($p<0.05$) and error frequency ($p<0.05$).

Table 9: Handgrip Results

Group	Test	Pre	Post
PRO(n=20)	**Handgrip** (MVC)	557±117	570±101
CON(n=20)	N.m	502±98	502±111
PRO	Squeeze Duration	83±56*	56±33*
CON	(s)	79±50*	51±32*
PRO	RPE	5.9±2.2*	6.9±1.8*
CON		5.6±2.0*	7.1±2.2*

Handgrip values. *significant main effect for time. MVC; maximum voluntary contraction, RPE; rate of perceived exertion. Values are means±SD.

Table 10: Vigilance Results

Group	Test	Pre	Post
PRO(n=12)	**Vigilance** (avg.Time)	0.33±0.04	0.35±0.04
CON(n=19)		0.33±0.05	0.32±0.04
PRO	#Correct	521±7	521±10
CON		519±13	518±11
PRO	#Incorrect	19±7	19±10
CON		21±13	22±11

Vigilance results. Values are means±SD

Table 11: Stroop Results

Group	Test	Pre	Post
PRO(n=19)	Speed (word/sec)	0.9±0.2*	1.0±0.2*
CON(n=19)		0.9±0.2*	1.0±0.2*
PRO	Incorrect Responses	7.5±5.5*	3.7±3.3*
CON		10.2±5.8*	6.6±4.7*
PRO	% Relative Error Frequency (error/word)	2.8±2.0*	1.2±1.0*
CON		4.1±3.0*	2.3±1.8*

PRO	Error Frequency (incorrect pre - incorrect post)	-	3.7±4.4
CON		-	3.6±3.9

Stroop values. *significant main effect for time. Values are means±SD.

Table 12. Operation Span Results

Group	Test	Pre	Post
PRO(n=20)	**Operation Span**	69±13	69±17
CON(n=19)		63±20	66±21

Operations Span values. Values are means±SD.

14. Discussion

The novel finding of the present study was that a higher protein-containing diet (PRO: 2.4 g/kg/d) consumed during a period of significant energy deficit (~40% reduction in estimated energy requirement) including high intensity training retained lean mass losses versus those seen in a group consuming a lower protein intake (CON: 1.2 g/kg/d). In fact, not only was lean mass loss retained in the PRO versus the CON group, but the PRO group actually showed an increase in lean mass during the same period. In addition, we observed a greater loss (~1.1kg) of fat mass in the PRO as compared with the CON group. While higher protein had a significant lean mass sparing effect it should be noted

that even during a period of high intensity exercise training performed during a period of significant energy deficit still resulted in lean mass preservation even when the amount of protein consumed (1.2g/kg/day) was relatively low and closer to what most young men consume habitually. Despite differences in body composition changes between groups we did not observe, as we hypothesized, differential responses in strength, performance measures, aerobic fitness, muscle cross-sectional area nor cognitive function or improved mood.

Several studies have looked at the impact of higher protein intakes and resistance exercise on retention of lean mass during energy deficit (7, 17, 18, 28, 77, 92); however, only one previous study has looked to combine both resistance exercise and higher protein during a marked energy deficit in what we would consider a 'completely' controlled manner: providing all macronutrient intake, and supervising workouts. Pasiakos et, al. (16) had subjects decrease their energy intake by 30% from required and had US military personnel undergo daily low-to-moderate-intensity (40-60% VO_{2peak}) treadmill and cycle ergometry as well as 3 days per week of lower intensity resistive type exercise (3 sets of 15 repetitions). Pasiakos' intervention lasted just 21 days and the group consuming the highest amount of protein (2.4g/kg/d) still lost 1.2±0.3kg of lean mass (16). In the present study both intervention groups trained until volitional failure in all exercises 6 d/wk and regardless of protein intake all groups retained (CON) or gained (PRO) lean mass. In the present study and that of Pasiakos et al (16) macronutrient intake was tightly controlled, creating personal nutritional prescriptions, and supplying all

meals. Pasiakos et al (16) included groups that consumed protein at three levels 0.8, 1.6, and 2.4g/kg/d of protein and they reported a significant retention of lean mass for the 1.6g/kg/d group, but no further retention in the 2.4g/kg/d. This finding is somewhat congruent with our observation that 1.2g/kg/d (75% of 1.6 g/kg/d) of dietary protein in the current study resulted in retention of lean mass; however, we report that provision of protein at 2.4g/kg/d actually supported lean mass gains, which was not seen by Pasiakos et al. (16). Thus, our data suggests that the exercise intensity, as opposed to total protein intake, may be an integral component of the resistance exercise and HIIT training in inducing lean mass gain versus merely net muscle mass retention. It is worth noting that our program of exercise, which involved an intense high volume resistance exercise and a HIIT regimen has not, at least to our knowledge, been studied in such a severe energy deficit.

The increased loss of fat mass in the current study was the sole contributor to the participants' weight loss. Data from Trapp et al. (40) suggests that lipolysis increases over 20 min of HIIT training gradually with each session. This research suggests the high intensities that the participants were training at likely enhanced their capacity for fat oxidation and may have induced an increase in muscle mitochondrial enzyme activity (40). Participants in the current study were considered recreationally active, and over weight (>25 BMI), which compared well to a recent study by Pasiakos et al. (16) (22-29 BMI & recreationally fit) supporting our data that a higher protein diet increases fat mass

loss. It has been suggested that athletes, with lower body fat, do not have a similar drop in fat mass with a higher protein intake at an energy deficit (7), but a well controlled trial has not, to our knowledge, been completed in an athletic population. Evidence from the current trial suggests that high quality weight loss (fat mass only), is attainable in a more controlled environment with higher intakes of dietary protein.

Our data suggests that during a significant energy deficit higher protein consumption (2.4g/kg/d) would have resulted in an increased stimulation of muscle protein synthesis or a suppression of proteolysis (or both) to a greater extent than consumption of 1.2g/kg/d as evidenced by gains in LM in the PRO group. Current evidence suggests that the energy deficit likely would have reduced basal MPS and reduced the sensitivity of MPS to feeding. Nonetheless, recent data have shown that lower MPS can also be 'rescued' by higher dietary protein (16, 17). Data from our laboratory (13) has shown that ~20-25g of protein (~0.25g protein/kg/meal) maximally stimulates MPS following resistance exercise. In the current study subjects in the PRO group would have regularly consumed ~ 49g protein/meal resulting in repeated periods of maximally stimulated MPS as compared with the CON group who consumed ~ 22g/meal. Importantly, however, the CON group also consumed enough protein, combined with anabolic exercise, throughout the intervention to retain their muscle mass despite a significant caloric deficit. Nevertheless, the consumption of higher protein (2.4g/kg/d) was able to induce lean mass accretion to a greater extent in the PRO group despite

significant caloric deficit. Clearly, the synergistic relationship between resistance/high intensity exercise and protein intake used in the present study served as a pillar for the retention and accretion of lean mass in the PRO and CON groups respectively (18).

As maintenance of lean mass was the primary goal of the current study, high quality, animal-source protein was used in both the PRO, and CON intervention groups. The PRO group received additional whey protein isolate in their study drinks, and higher protein content meals whereas the CON group received milk, chicken, and other meat proteins as their daily protein sources. Data for our lab (17, 77, 93), has shown the increased anabolic response to resistance exercise being greater when an animal-source protein is consumed. With a higher quality, and timed protein being consumed during each feeding it is likely that a rapid post-exercise aminoacidemia was a main contributor to the increased retention, and accretion of lean mass. We propose that protein quality and protein timing relative to exercise would become more important when in a caloric deficit since in the absence of timed quality protein consumption other comparable studies have reported declines in lean mass while subjects were in an energy deficit (7, 16, 73).

It is likely that the differing fat content, as versus carbohydrate, between the PRO and CON energy-restricted diets of our subjects allowed each group to train at similar levels throughout the intervention. Carbohydrate consumption was equivalent between groups and this would presumably have resulted in glycogen

levels being replenished similarly. With participants training at or near maximal intensity during each supervised visit, CHO would have to have been the main fuel-source oxidized during the types of exercise we employed. Supplementation of protein and CHO in post-workout drinks, which were consumed by participants under supervision would have, we propose, have allowed for equivalent recovery in so far as glycogen replenishment is concerned, despite the high exercise intensities and substantial energy deficit.

We observed significant increases in performance as a result of our training intervention, however, there were no differences in any of the measures of performance between groups. Thus our findings are not in agreement with our original hypothesis that with an increased amount of lean mass preservation in the PRO group we would see greater strength gains when compared to CON. However, the difference in lean mass between the groups while statistically significant was not large (~1.1kg). In addition, with equal fuel stores and training intensities between groups it is perhaps not surprising that strength measure gains were not different between groups. Our data is in agreement with a previous study showing no benefit of increased protein intake on strength or anaerobic performance measures during energy deficit (7). However, if substantial strength gains were the goal of the present study then lower repetition, higher weight resistance programs would be better suited as they are superior at inducing gains in strength (20). While the main goal of the present study was to preserve lean mass during weight loss it is important to note that

both groups experienced significant gains in strength and performance despite training in a significant energy deficit. Our findings are in line with those of others (7, 94) who showed that high-intensity resistance training programs can induce substantial strength gains. The improved performance seen in both groups in the current study is concordant with results from recent studies from Gibala et al, (35) showing that HIIT training increases aerobic capacity, and improves time trial performance. Participants in the present study showed decreased time trial times and increased their $VO_{2\ peak}$ over the intervention (35).

In the current study circuit-type resistance training was performed to failure and resulted in increased muscle fibre CSA, which further solidifies notions that full muscle fibre recruitment, and not merely performance of high load contractions, is a fundamental variable underpinning resistance exercise-induced MPS rates (20). Although resistance training was not the only method of training in this study, it is seen as the most potent stimulus of MPS and hypertrophy (17). We have also reported that the higher volume of repetitions involved with lifting a lower weight to failure (circuit-type training in the current study) seems to promote MPS rates for a longer duration (22-24hrs) (19). It is possible that the HIIT training further contributed to increases in type 2 muscle fibre CSA (95), although recent evidence suggests otherwise (96). The high exercise intensities that are induced by HIIT may induce a rapid remodeling of skeletal muscle, due to the high level of muscle fibre recruitment involved, but may be a minor contributor to hypertrophy as versus resistance exercise (35, 96).

In the current study mood and cognitive measures were taken as a secondary measurement to look at the psychological effects of the combination of energy deficit and hybrid exercise methods in a very regimented and controlled intervention that to our knowledge is unprecedented. Data suggests that a severe energy deficit, apart from exercise, can negatively affect cognitive performance (47, 50-52). Redman et al. (47) suggests this is due to the pre-occupation with body weight and food. The mind has obsessive thoughts about eating and weight (47, 52). Green et al (52) reported that these thoughts place demand on limited cognitive performance, leaving less available for performance on cognitive tasks. Our data is consistent with studies completed by Bryan et al. (49) and Kretsch et al (97) where a weight loss diet was prescribed to over-weight women and showed little impact on cognitive performance, both suggesting that being on a prescribed diet lessens the drain of cognitive resources as opposed to self-imposed diets and their usual decrements in cognition. Exercise has been shown to only significantly improve cognitive performance at light, light (60), or moderate (67) intensities. The current study included high intensity training and therefore is in line with previous findings (60, 67) showing no change in processing speed (vigilance task), or executive function (OSPAN). Performance on the Stroop task showed a significant increase for time in both the PRO and CON groups, with significantly less errors ($p<0.05$). This was also found in a study by Kretsch et al (97) where over weight women recalled words at a faster rate, but showed no other improvements in their cognitive performance while at an energy deficit.

Our data in the handgrip tasks suggest that cognitive performance may have been compromised, showing a significant reduction in squeeze duration, and increase in RPE. Although there is a physical component to this test, the specific isometric contraction involved was not trained throughout the intervention, leading to decrements in self-control.

When looking at mood, intensity may have played a prominent role in significant decreases in positive affect ($p<0.05$). It is well documented that exercise increases mood and physical well being (54-58), but it is also known that at a severe energy deficit mood can be negatively affected (50, 98, 99). With a 40% reduction in macronutrient intake, a considerable deficit, exercise didn't seem to negate decreases in positive affect. This suggesting that high intensity exercise does not increase positive affect which has been previously shown (59). This is in line with additional similar research by Mettler et al (7) where reduced feelings of well-being were documented during an energy restricted, resistance training trial in athletes. However, the reduction in PA was found in both intervention groups in the current study, and just in the high protein group by Mettler (7). Additionally significant increases in fatigue ($p<0.05$) shown in the POMS for both groups in the current study, is only evident in the higher protein group for the Mettler intervention (7). It is possible again that the higher intensity training program in the current study was a key contributor to increased fatigue regardless of the energy-restricted diets. A slight decrease in fatigue by the end

of the current study suggests that participants began to become acclimatized to the high amount of effort involved.

In summary, the present study provides evidence that consuming a higher protein diet (2.4g/kg/d) during energy deficit (~40% reduction in energy intake versus requirements) while performing intense resistance exercise training and HIIT, can augment lean mass over a 28-day period. Furthermore, a high-intensity resistance plus HIIT workout performed during energy deficit preserves lean mass even at a lower protein intake (1.2g/kg/d). This study also provides further support that exercise intensity and volume leading to a higher fibre recruitment is imperative for muscle hypertrophy (or preservation of muscle fibre size) as evidenced by the muscle fibre CSA adaptations. However, evidence to suggest that psychological fitness grows with physical fitness at an energy deficit needs to be further reviewed. In conclusion, the current study provides direct evidence that a higher protein diet during significant energy deficit and high intensity training not only preserves, but increases, lean mass and high intensity training during energy deficit, irrespective of protein intake, increases strength and performance in young men.

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APPENDIX 1

SUBJECT ANTHROPOMETRIC CHARACTERISTICS

Subject	Group	Height (m)	Weight (kg)	Age (yr)	BMI (kg/m^2)
R1	PRO	1.87	84	22	27.0
R2	PRO	1.83	87	21	25.2
R4	PRO	1.75	97	21	28.5
R5	PRO	1.92	95	22	26.4
R6	PRO	1.88	99	24	26.8
R13	PRO	1.77	108	23	31.7
R16	PRO	1.84	121	22	35.9
R17	PRO	1.86	120	22	31.2
R19	PRO	1.93	124	23	32.2
R21	PRO	1.80	107	23	38.3
R43	PRO	1.76	103	22	34.6
R24	PRO	1.92	91	21	27.9
R26	PRO	1.85	120	28	26.6
R27	PRO	1.82	96	22	36.2
R30	PRO	1.89	85	23	26.9
R31	PRO	1.80	101	22	26.2
R33	PRO	1.85	87	22	29.4
R38	PRO	1.85	94	24	25.3
R39	PRO	1.80	88	23	28.9
R40	PRO	1.73	84	26	29.5

	MEAN	1.84	100.1	22.8	
	SD	0.06	12.8	1.7	
R3	CON	1.74	85	22	28.1
R8	CON	1.73	86	20	28.9
R9	CON	1.90	105	20	29.0
R10	CON	1.65	85	21	31.2
R11	CON	1.78	94	23	29.6
R12	CON	1.71	79	23	27.1
R14	CON	1.75	82	23	26.8
R18	CON	1.93	111	23	29.9
R20	CON	1.75	79	22	25.8
R23	CON	1.73	91	19	30.3
R25	CON	1.85	98	27	28.6
R28	CON	1.98	105	21	26.7
R29	CON	1.74	86	21	28.4
R32	CON	1.88	129	20	36.6
R34	CON	1.80	105	30	32.5
R35	CON	1.62	77	22	29.2
R36	CON	1.85	95	22	27.7
R37	CON	1.83	103	23	30.6
R41	CON	1.85	102	27	29.9

R42	CON	1.86	123	22	35.7
	MEAN	1.80	96.0	22.6	
	SD	0.09	14.6	2.7	

APPENDIX 2

FOUR COMPARTMENT MODEL RESULTS AND ANOVA TABLES

FOUR COMPARTMENT MODEL

GROUP	MEASURE	TIME	MEAN	SD	SE
PRO	Lean Mass (kg)	Pre	73.0	6.82	1.68
		Post	74.1	6.74	1.64
CON		Pre	69.2	8.16	1.68
		Post	69.2	7.85	1.64
PRO	Fat Mass (kg)	Pre	23.6	5.56	1.30
		Post	19.0	6.26	1.39
CON		Pre	24.7	6.04	1.30
		Post	21.1	6.12	1.39

FOUR COMPARTMENT MODEL ANOVA RESULTS

LEAN MASS

WITHIN-SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	6.102	6.102	5.554	0.024
time x group	1	4.721	4.721	4.296	0.045
Error(time)	38	41.751	1.099		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	407506.000	407506.000	3741.468	0.000
group	1	375.225	375.225	3.445	0.071
Error	38	4138.811	108.916		

TUKEY POST HOC

MEASURE	TIME	P
PRO	Pre vs. Post	0.003
CON	Pre vs. Post	0.842
Pre	PROvs.CON	0.114
Post	PROvs.CON	0.044

Paired T-Test difference between groups – P=0.04502516

FAT MASS

Source of Variation	DF	SS	MS	F	P
time	1	331.897	331.897	285.452	0.000
time x group	1	5.841	5.841	5.024	0.031
Error(time)	38	44.183	1.163		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	39073.719	39073.719	551.024	0.000
group	1	49.557	49.557	0.699	0.408
Error	38	2694.621	70.911		

TUKEY POST HOC

MEASURE	TIME	P
PRO	Pre vs. Post	0.000
CON	Pre vs. Post	0.000
Pre	PROvs.CON	0.577
Post	PROvs.CON	0.287

Paired T-Test difference between groups – P=0.0309196

APPENDIX 3

STRENGTH RESULTS AND ANOVA TABLES

STRENGTH MEASURES

GROUP	MEASURE	TIME	MEAN	SD	SE
PRO	Leg Press (kg)	Pre	377	60	35.830
		Post	439	78	36.362
CON		Pre	337	70	35.830
		Post	397	71	36.362
PRO	Bench Press (kg)	Pre	107	29	11.565
		Post	112	25	11.049
CON		Pre	89	16	11.565
		Post	97	19	11.049
PRO	Push Up	Pre	29	12	2.472
		Post	39	10	2.594
CON		Pre	24	10	2.472
		Post	31	12	2.594
PRO	Sit Up	Pre	36	9	2.323
		Post	47	8	2.366
CON		Pre	33	12	2.323
		Post	40	13	2.366
PRO	Biodex (N.m)	Pre	329	59	12.426
		Post	336	67	13.496
CON		Pre	294	52	12.426
		Post	291	53	13.496

STRENGTH MEASURES ANOVA RESULTS

LEG PRESS

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	339822.450	339822.450	88.444	0.000
time x group	1	480.200	480.200	0.125	0.726
Error(time)	38	146005.350	3842.246		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	58800492.4	58800492.4	1217.967	0.000
group	1	480.200	480.200	2.792	0.103
Error	38	146005.350	48277.562		

BENCH PRESS

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	3962.112	3962.112	27.865	0.000
time x group	1	374.113	374.113	2.631	0.113
Error(time)	38	5403.275	142.191		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	3968068.61	3968068.61	797.689	0.000
group	1	27121.612	27121.612	5.452	0.025
Error	38	189029.275	4974.455		

PUSH UP

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	1411.200	1411.200	128.352	0.000
time x group	1	20.000	20.000	1.819	0.185
Error(time)	38	417.800	10.995		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	77625.800	77625.800	315.789	0.000
group	1	696.200	696.200	2.832	0.101
Error	38	9341.000	245.816		

SIT UP

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	1739.112	1739.112	122.524	0.000
time x group	1	78.012	78.012	5.496	0.240
Error(time)	38	539.375	14.194		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	122539.512	122539.512	595.828	0.000
group	1	427.812	427.812	2.080	0.157
Error	38	7815.175	205.662		

BIODEX

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	80.000	80.000	0.211	0.648
time x group	1	470.450	470.450	1.244	0.272
Error(time)	38	14374.550	378.278		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	7820001.80	7820001.80	1230.972	0.000
group	1	31760.450	31760.450	5.000	0.031
Error	38	241402.750	6352.704		

APPENDIX 4

CYCLE ERGOMETER RESULTS AND ANOVA TABLES

CYCLE ERGOMETER RESULTS

GROUP	MEASURE	TIME	MEAN	SD	SE
PRO	Peak Power (W)	Pre	1148	130	44.405
		Post	1277	133	38.647
CON		Pre	1095	249	44.405
		Post	1237	205	38.647
PRO	Mean Power (W)	Pre	768	76	17.866
		Post	805	76	19.718
CON		Pre	707	83	17.866
		Post	743	98	19.718
PRO	Total Work (J)	Pre	23029	2295	559.812
		Post	24104	2271	593.229
CON		Pre	20883	2696	559.812
		Post	22237	2986	593.229
PRO	VO_2 Max (ml/kg/min)	Pre	41.1	5.6	1.182
		Post	47.2	7.8	1.601
CON		Pre	40.5	4.9	1.182
		Post	44.6	6.4	1.601

TIME TRIAL

GROUP	MEASURE	TIME	MEAN	SD	SE
PRO	Time Trial (min:sec)	Wk1	19:09	3:54	00:45
		Wk2	17:03	3:16	00:45
		Wk3	16:33	3:20	00:46
		Wk4	15:37	2:53	00:56
CON		Wk1	21:13	3:43	00:45
		Wk2	19:03	2:44	00:45
		Wk3	18:03	2:44	00:46
		Wk4	17:09	2:22	00:56

CYCLE ERGOMETER ANOVA RESULTS

PEAK POWER

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	366663.200	366663.200	49.592	0.000
time x group	1	994.050	994.050	0.134	0.716
Error(time)	38	280956.750	7393.599		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	113159516	113159516	1827.698	0.000
group	1	42504.200	42504.200	0.687	0.413
Error	38	2352719.35	61913.667		

MEAN POWER

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	27121.613	27121.613	13.143	0.001
time x group	1	10.513	10.513	0.005	0.943
Error(time)	38	78413.375	2063.510		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	45742538.1	45742538.1	3781.388	0.000
group	1	75706.513	75706.513	6.258	0.017
Error	38	459676.875	12096.760		

TOTAL WORK

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	27154986.0	27154986.0	14.001	0.001
time x group	1	160294.512	160294.512	0.083	0.775
Error(time)	38	73701496.0	1939513.05		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	4.082E+10	4.082E+10	3591.101	0.000
group	1	76563714.6	76563714.6	6.736	0.013
Error	38	431933934	11366682.5		

VO_2 MAX

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	514.605	514.605	61.511	0.000
time x group	1	18.528	18.528	2.215	0.145
Error(time)	38	317.912	8.366		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	150242.445	150242.445	2121.880	0.000
group	1	53.301	53.301	0.753	0.391
Error	38	2690.639	70.806		

TIME TRIAL

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
Time					
Linear	1	1089583.22	1089583.22	107.103	0.000
Quadratic	1	48511.225	48511.225	14.322	0.001
Cubic	1	18412.805	18412.805	3.330	0.076
time x group					
Linear	1	10628.820	10628.820	1.045	0.313

Quadratic	1	3.025	3.025	0.001	0.976
Cubic	1	1295.405	1295.405	0.234	0.631
Error(time)					
Linear	38	386584.160	10173.267		
Quadratic	38	128710.750	3387.125		
Cubic	38	210103.590	5529.042		

BETWEEN-SUBJECT EFFECTS

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Intercept	1	186002438	186002438	1493.648	0.000
group	1	438902.500	438902.500	3.525	0.068
Error	38	4732100.10	124528.950		

POST HOC ANALYSIS

MEASURE	**TIME**	**P**
Time Trial	wk1 vs. wk2	0.000
	wk1 vs. wk3	0.000
	wk1 vs. wk4	0.000
	wk2 vs. wk3	0.005
	wk2 vs. wk4	0.000
	wk3 vs. wk4	0.000

APPENDIX 5

CROSS SECTIONAL AREA RESULTS AND ANOVA TABLES

CROSS SECTIONAL AREA RESULTS

GROUP	MEASURE	TIME	MEAN	SD	SE
PRO	Type 1 CSA	Pre	5374.7	1260.7	386.740
		Post	6109.8	1981.8	429.385
CON		Pre	5555.0	1281.4	376.947
		Post	5739.4	1232.1	418.513
PRO	Type 2 CSA	Pre	6789.6	1353.7	398.593
		Post	7638.6	2961.3	568.484
CON		Pre	6855.6	1635.4	388.501
		Post	7375.0	1681.1	554.089
PRO	Total CSA	Pre	6082.2	1476.0	372.749
		Post	6874.2	2603.3	490.726
CON		Pre	6205.3	1592.7	363.311
		Post	6557.2	1674.0	478.301

CROSSE SECTIONAL AREA ANOVA RESULTS

TYPE 1 CSA

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	3994555.21	3994555.21	2.779	0.104
time x group	1	1402796.23	1402796.23	0.976	0.330
Error(time)	37	53179474.9	1437283.11		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	2.670E+9	2.670E+9	544.119	0.000
group	1	3300799.84	3300799.84	0.673	0.417
Error	37	181579714	4907559.83		

TYPE 2 CSA

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	9057485.77	9057485.77	4.034	0.050
time x group	1	514625.001	514625.001	0.229	0.635
Error(time)	37	83065659.7	2245017.83		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	4.183E+9	4.183E+9	604.972	0.000
group	1	3436956.95	0.497	0.497	0.485
Error	37	255815739			

TOTAL CSA

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	6910936.31	6910936.31	4.101	0.050
time x group	1	596192.844	596192.844	0.354	0.556
Error(time)	37	62353268.8	1685223.48		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	3.548E+9	3.548E+9	641.650	0.000
group	1	3154638.17	3154638.17	0.570	0.455
Error	37	204613955	5530106.88		

APPENDIX 6

POMS RESULTS AND ANOVA TABLES

POMS RESULTS

POMS

GROUP	MEASURE	TIME	MEAN	SD	SE
PRO	Depression	Pre	0.29	0.57	0.135
		Wk1	0.43	0.67	0.145
		Wk2	0.27	0.29	0.094
		Wk3	0.26	0.30	0.117
		Wk4	0.29	0.45	0.170
CON		Pre	0.33	0.65	0.147
		Wk1	0.39	0.64	0.158
		Wk2	0.29	0.53	0.102
		Wk3	0.32	0.70	0.127
		Wk4	0.52	1.01	0.184
PRO	Vigor	Pre	2.32	0.57	0.121
		Wk1	2.19	0.75	0.174
		Wk2	2.13	0.75	0.185
		Wk3	2.11	0.80	0.180
		Wk4	2.22	0.87	0.193
CON		Pre	1.72	0.50	0.132
		Wk1	1.97	0.81	0.189
		Wk2	2.01	0.91	0.201
		Wk3	1.94	0.81	0.195

		Wk4	2.04	0.85	0.209
PRO	Confusion	Pre	0.78	0.63	0.142
		Wk1	0.73	0.65	0.156
		Wk2	0.58	0.51	0.131
		Wk3	0.47	0.58	0.159
		Wk4	0.47	0.68	0.160
CON		Pre	0.79	0.64	0.154
		Wk1	0.82	0.75	0.169
		Wk2	0.73	0.67	0.142
		Wk3	0.60	0.84	0.172
		Wk4	0.61	0.76	0.173
PRO	Tension	Pre	0.80	0.58	0.150
		Wk1	0.78	0.60	0.150
		Wk2	0.55	0.50	0.128
		Wk3	0.53	0.50	0.139
		Wk4	0.49	0.56	0.165
CON		Pre	0.89	0.77	0.162
		Wk1	0.99	0.75	0.163
		Wk2	0.69	0.65	0.139
		Wk3	0.73	0.74	0.151
		Wk4	0.75	0.91	0.179
PRO	Anger	Pre	0.34	0.52	0.127

		Wk1	0.53	0.57	0.132
		Wk2	0.39	0.47	0.112
		Wk3	0.24	0.38	0.124
		Wk4	0.21	0.31	0.138
CON		Pre	0.45	0.62	0.138
		Wk1	0.55	0.62	0.144
		Wk2	0.51	0.54	0.121
		Wk3	0.54	0.71	0.135
		Wk4	0.47	0.85	0.150
PRO	Fatigue	Pre	0.96	0.79	0.178
		Wk1	1.75	0.84	0.194
		Wk2	1.40	0.63	0.146
		Wk3	1.43	0.90	0.182
		Wk4	1.07	0.95	0.220
CON		Pre	1.06	0.81	0.193
		Wk1	1.82	0.90	0.210
		Wk2	1.60	0.68	0.159
		Wk3	1.31	0.80	0.197
		Wk4	1.28	1.02	0.238

POMS ANOVA RESULTS

DEPRESSION

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
Time					
Linear	1	0.016	0.016	0.060	0.808
Quadratic	1	0.072	0.072	0.520	0.476
Cubic	1	0.392	0.392	2.777	0.105
Order 4	1	0.088	0.088	1.603	0.214
time x group					
Linear	1	0.215	0.215	0.818	0.372
Quadratic	1	0.138	0.138	0.996	0.325
Cubic	1	0.000	0.000	0.001	0.970
Order 4	1	0.015	0.015	0.275	0.603
Error(time)					
Linear	35	9.194	0.263		
Quadratic	35	4.867	0.139		
Cubic	35	4.936	0.141		
Order 4	35	1.919	0.055		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	21.272	21.272	17.488	0.000
group	1	0.176	0.176	0.145	0.706
Error	35	42.573	1.216		

VIGOR

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
Time					
Linear	1	0.098	0.098	0.402	0.530
Quadratic	1	0.005	0.005	0.018	0.895
Cubic	1	0.165	0.165	1.069	0.308
Order 4	1	0.010	0.010	0.070	0.794
time x group					
Linear	1	0.760	0.760	3.131	0.086
Quadratic	1	0.568	0.568	2.025	0.164
Cubic	1	0.107	0.107	0.690	0.412
Order 4	1	0.000	0.000	0.003	0.956
Error(time)					
Linear	35	8.492	0.243		
Quadratic	35	9.812	0.280		

Cubic	35	5.409	0.155		
Order 4	35	4.999	0.143		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	784.440	784.440	363.467	0.000
group	1	3.139	3.139	1.454	0.236
Error	35	75.537	2.158		

CONFUSION

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
Time					
Linear	1	1.949	1.949	7.420	0.010
Quadratic	1	0.002	0.002	0.010	0.919
Cubic	1	0.212	0.212	1.651	0.207
Order 4	1	2.003E-005	2.003E-005	0.000	0.989
time x group					
Linear	1	0.085	0.085	0.322	0.574
Quadratic	1	0.032	0.032	0.155	0.696
Cubic	1	0.003	0.003	0.026	0.872
Order 4	1	0.003	0.003	0.031	0.862

Error(time)					
Linear	35	9.194	0.263		
Quadratic	35	7.332	0.209		
Cubic	35	4.499	0.129		
Order 4	35	3.456	0.099		

BETWEEN-SUBJECT EFFECTS

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Intercept	1	79.643	79.643	51.440	0.000
group	1	0.503	0.503	0.325	0.572
Error	35	54.189	1.548		

POST HOC ANALYSIS

MEASURE	**TIME**	**P**
Confusion	Pre vs. wk1	0.944
	Pre vs. wk2	0.200
	Pre vs. wk3	0.020
	Pre vs. wk4	0.038
	wk1 vs. wk2	0.136
	wk1 vs. wk3	0.013
	wk1 vs. wk4	0.050
	wk2 vs. wk3	0.119

	wk2 vs. wk4	0.262
	wk3 vs. wk4	0.946

TENSION

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
Time					
Linear	1	1.889	1.889	5.475	0.025
Quadratic	1	0.086	0.086	0.752	0.392
Cubic	1	0.321	0.321	2.051	0.161
Order 4	1	0.402	0.402	5.149	0.030
time x group					
Linear	1	0.092	0.092	0.266	0.609
Quadratic	1	8.343E-005	8.343E-005	0.001	0.979
Cubic	1	0.028	0.028	0.178	0.676
Order 4	1	0.029	0.029	0.366	0.549
Error(time)					
Linear	35	12.077	0.345		
Quadratic	35	3.990	0.114		
Cubic	35	5.470	0.156		
Order 4	35	2.731	0.078		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	94.988	94.988	64.829	0.000
group	1	1.453	1.453	0.992	0.326
Error	35	51.283	1.465		

POST HOC ANALYSIS

MEASURE	TIME	P
Tension	Pre vs. wk1	0.675
	Pre vs. wk2	0.015
	Pre vs. wk3	0.012
	Pre vs. wk4	0.109
	wk1 vs. wk2	0.001
	wk1 vs. wk3	0.006
	wk1 vs. wk4	0.031
	wk2 vs. wk3	0.918
	wk2 vs. wk4	0.998
	wk3 vs. wk4	0.937

ANGER

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
Time					
Linear	1	0.218	0.218	0.827	0.369
Quadratic	1	0.354	0.354	4.857	0.340
Cubic	1	0.222	0.222	1.979	0.168
Order 4	1	0.036	0.036	0.442	0.510
time x group					
Linear	1	0.299	0.299	1.137	0.294
Quadratic	1	0.021	0.021	0.291	0.593
Cubic	1	0.153	0.153	1.361	0.251
Order 4	1	0.004	0.004	0.045	0.834
Error(time)					
Linear	35	9.208	0.263		
Quadratic	35	2.551	0.073		
Cubic	35	3.935	0.112		
Order 4	35	2.841	0.081		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	32.836	32.836	30.204	0.000
group	1	1.171	1.171	1.077	0.306
Error	35	38.049	1.087		

FATIGUE

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
Time					
Linear	1	0.027	0.027	0.048	0.827
Quadratic	1	8.352	8.352	14.628	0.001
Cubic	1	3.708	3.708	11.412	0.002
Order 4	1	1.079	1.079	3.990	0.054
time x group					
Linear	1	0.001	0.001	0.001	0.970
Quadratic	1	0.049	0.049	0.086	0.772
Cubic	1	0.238	0.238	0.732	0.398
Order 4	1	0.385	0.385	1.426	0.240
Error(time)					
Linear	35	19.340	0.553		
Quadratic	35	19.982	0.571		

Cubic	35	11.373	0.325		
Order 4	35	9.462	0.270		

BETWEEN-SUBJECT EFFECTS

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Intercept	1	343.967	343.967	200.145	0.000
group	1	0.390	0.390	0.227	0.637
Error	35	60.151	1.719		

POST HOC ANALYSIS

MEASURE	**TIME**	**P**
Fatigue	Pre vs. wk1	0.000
	Pre vs. wk2	0.004
	Pre vs. wk3	0.009
	Pre vs. wk4	0.318
	wk1 vs. wk2	0.025
	wk1 vs. wk3	0.006
	wk1 vs. wk4	0.002
	wk2 vs. wk3	0.374
	wk2 vs. wk4	0.090
	wk3 vs. wk4	0.096

APPENDIX 7

PANAS RESULTS AND ANOVA TABLES

PANAS RESULTS

GROUP	MEASURE	TIME	MEAN	SD	SE
PRO	Positive Affect	Pre	35.9	6.79	1.676
		Wk1	33.5	7.92	1.870
		Wk2	33.0	7.76	1.737
		Wk3	32.2	8.73	1.920
		Wk4	35.6	10.4	2.111
CON		Pre	30.3	8.25	1.817
		Wk1	30.7	8.85	2.028
		Wk2	29.4	7.78	1.884
		Wk3	28.4	8.40	2.082
		Wk4	31.7	8.18	2.290
PRO	Negative Affect	Pre	13.6	3.46	0.920
		Wk1	13.0	2.72	0.982
		Wk2	11.9	2.58	0.855
		Wk3	11.7	2.27	0.910
		Wk4	11.5	2.35	1.073
CON		Pre	13.7	4.78	0.998
		Wk1	13.7	5.78	1.065
		Wk2	13.1	4.91	0.927
		Wk3	13.2	5.49	0.987
		Wk4	13.8	6.62	1.164

PANAS ANOVA RESULTS

POSITIVE AFFECT

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
Time					
Linear	1	2.318	2.318	0.816	0.816
Quadratic	1	195.112	195.112	0.008	0.008
Cubic	1	61.655	61.655	0.065	0.065
Order 4	1	9.024	9.024	0.266	0.266
time x group					
Linear	1	5.345	5.345	0.724	0.724
Quadratic	1	18.849	18.849	0.386	0.386
Cubic	1	12.520	12.520	0.396	0.396
Order 4	1	2.552	2.552	0.552	0.552
Error(time)					
Linear	35	1476.812	42.195		
Quadratic	35	854.367	24.410		
Cubic	35	593.269	16.951		
Order 4	35	247.421	7.069		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	188866.328	188866.328	730.325	0.000
group	1	708.144	708.144	2.738	0.107
Error	35	9051.207	258.606		

POST HOC ANALYSIS – POSITIVE AFFECT

MEASURE	TIME	P
PA	Pre vs. wk1	0.351
	Pre vs. wk2	0.042
	Pre vs. wk3	0.013
	Pre vs. wk4	0.683
	wk1 vs. wk2	0.246
	wk1 vs. wk3	0.181
	wk1 vs. wk4	0.350
	wk2 vs. wk3	0.350
	wk2 vs. wk4	0.055
	wk3 vs. wk4	0.000

NEGATIVE AFFECT

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
Time					
Linear	1	29.518	29.518	1.975	0.619
Quadratic	1	8.108	8.108	1.504	0.228
Cubic	1	1.266	1.266	0.443	0.510
Order 4	1	2.600	2.600	1.320	0.258
time x group					
Linear	1	27.118	27.118	1.815	0.187
Quadratic	1	0.000	0.000	0.000	0.996
Cubic	1	0.206	0.206	0.072	0.790
Order 4	1	0.027	0.027	0.013	0.908
Error(time)					
Linear	35	523.071	14.945		
Quadratic	35	188.629	5.389		
Cubic	35	99.902	2.854		
Order 4	35	68.928	1.969		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	30565.332	30565.332	468.339	0.000
group	1	63.149	63.149	0.968	0.332
Error	35	2284.214	65.263		

APPENDIX 8

COGNITIVE FUNCTION RESULTS AND ANOVA TABLES

COGNITIVE FUNCTION RESULTS

GROUP	MEASURE	TIME	MEAN	SD	SE
PRO	**HANDGRIP** Peak MVC	Pre	557.5	116.8	24.080
		Post	570.4	101.1	23.754
CON		Pre	502.1	97.7	24.080
		Post	502.0	111.2	23.754
PRO	Squeeze Duration (secs)	Pre	83.4	56.4	11.879
		Post	56.1	33.1	7.236
CON		Pre	78.6	49.6	11.879
		Post	50.7	31.5	7.236
PRO	RPE	Pre	5.9	2.2	0.483
		Post	6.9	1.8	0.449
CON		Pre	5.6	2.0	0.483
		Post	7.1	2.2	0.449
PRO	**VIGILANCE** Avg. Time	Pre	0.328	0.037	0.014
		Post	0.346	0.037	0.012
CON		Pre	0.328	0.050	0.011
		Post	0.322	0.043	0.009
PRO	#Right	Pre	520.9	7.10	3.116
		Post	520.7	10.0	2.994
CON		Pre	519.2	12.5	2.476
		Post	517.8	10.6	2.379

PRO	#Wrong	Pre	19.1	7.10	3.116
		Post	19.3	10.0	2.994
CON		Pre	20.8	12.5	2.476
		Post	22.1	10.6	2.379
PRO	**STROOP** Speed (word/sec)	Pre	0.906	0.190	0.0158
		Post	1.018	0.189	0.0264
CON		Pre	0.908	0.182	0.0158
		Post	0.998	0.179	0.0264
PRO	Error Frequency	Pre/Post	3.74	4.42	1.014
		-	-	-	-
CON		Pre/Post	3.58	3.93	0.903
		-	-	-	-
PRO	Relative Error Frequency (%)	Pre	2.8	2.0	0.572
		Post	1.2	1.0	0.338
CON		Pre	4.1	3.0	0.572
		Post	2.3	1.8	0.338
PRO	#Wrong	Pre	7.5	5.5	1.301
		Post	3.7	3.3	0.937
CON		Pre	10.2	5.8	1.301
		Post	6.6	4.7	0.937
PRO	**Operation Span**	Pre	69.4	13	3.808
		Post	69.2	16.7	4.247

CON		Pre	62.5	20.4	3.906
		Post	66.2	21.1	4.357

COGNITIVE FUNCTION ANOVA TABLES

HANDGRIP

PEAK MVC

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	819.324	819.324	0.630	0.432
time x group	1	842.261	842.261	0.647	0.426
Error(time)	38	49448.408	1301.274		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	22729604.1	22729604.1	1053.255	0.000
group	1	76621.057	76621.057	3.551	0.067
Error	38	820053.021	21580.343		

SQUEEZE DURATION

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	15218.351	15218.351	11.651	0.002
time x group	1	1.906	1.906	0.001	0.970
Error(time)	38	49633.493	1306.145		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	361905.798	361905.798	141.179	0.000
group	1	518.623	518.623	0.202	0.655
Error	38	97411.165	2563.452		

RPE

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	37.812	37.812	43.443	0.000
time x group	1	0.612	0.612	0.704	0.407
Error(time)	38	33.075	0.870		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	3289.612	3289.612	419.798	0.000
group	1	0.112	0.112	0.014	0.905
Error	38	297.775	7.836		

VIGILANCE

AVG. TIME

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	0.000	0.000	1945.028	0.000
time x group	1	0.002	0.002	0.626	0.435
Error(time)	29	0.018	0.001		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	6.451	6.451	1945.028	0.000
group	1	0.002	0.002	0.626	0.435
Error	29	0.096	0.003		

#RIGHT

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	9.632	9.632	0.325	0.573
time x group	1	4.600	4.600	0.155	0.696
Error(time)	29	859.336	29.632		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	15889126.8	15889126.8	81729.378	0.000
group	1	75.487	75.487	0.388	0.538
Error	29	5637.932	194.411		

#WRONG

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	254.224	254.224	29.040	0.000
time x group	1	0.118	0.118	0.014	0.908
Error(time)	29	315.158	8.754		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	24344.907	24344.907	125.224	0.000
group	1	75.487	75.487	0.388	0.538
Error	29	5637.932	194.411		

STROOP

SPEED (word/sec)

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	0.193	0.193	42.901	0.000
time x group	1	0.002	0.002	0.493	0.487
Error(time)	36	0.162	0.004		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	69.639	69.639	1085.974	0.000
group	1	0.002	0.002	0.025	0.876
Error	36	2.309	0.064		

ERROR FREQUENCY (one-way ANOVA)

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.237	0.237	0.014	0.908
Within Groups	36	630.316	17.509		
Total	37	630.553			

RELATIVE ERROR FREQUENCY

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	51.351	51.351	35.971	0.000
time x group	1	0.147	0.147	0.103	0.750
Error(time)	36	51.393	1.428		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	518.501	518.501	74.554	0.000
group	1	27.083	27.083	3.894	0.056
Error	36	250.370	6.955		

#WRONG

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	254.224	254.224	29.040	0.000
time x group	1	0.118	0.118	0.014	0.908
Error(time)	36	315.158	8.754		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	3738.013	3738.013	93.266	0.000
group	1	150.645	150.645	3.759	0.060
Error	36	1442.842	40.079		

OPERATION SPAN

WITHIN SUBJECTS CONTRASTS

Source of Variation	DF	SS	MS	F	P
time	1	56.205	56.205	0.430	0.516
time x group	1	72.835	72.835	0.558	0.460
Error(time)	37	4832.653	130.612		

BETWEEN-SUBJECT EFFECTS

Source of Variation	DF	SS	MS	F	P
Intercept	1	348007.470	348007.470	669.163	0.000
group	1	475.583	475.583	0.914	0.345
Error	37	19242.363	520.064		