The Effects of High Protein Intakes During Energy Restriction on Body Composition, Energy Metabolism and Physical Performance in Athletes

Mikael Kanaan

Thesis submitted to the University of Ottawa
in partial fulfillment of the requirements for the
degree of Master of Science
in Human Kinetics

School of Human Kinetics
Faculty of Health Sciences
University of Ottawa

Abstract

Introduction

Weight loss practices are commonly used by athletes who perceive lower levels of body fat and higher fat-free mass (FFM) as advantageous for physical performance. High protein diets have been associated with greater fat loss and greater retention of lean mass during periods of energy restriction (ER) in individuals with obesity. However, less is known about the effects of high protein diets in trained individuals during ER. It has been proposed that leaner individuals are more at risk of losing FFM during ER than individuals with overweight. The current ACSM recommendation for protein intake in active individuals is 1.2-1.7g/kg. However, it is unclear whether higher intakes are necessary to maximize FFM retention and maximize physical performance in athletes undergoing periods of ER.

Objective

Our primary objective was to determine whether high protein intakes could maximize FFM retention in trained individuals during periods of ER. We also aimed to determine whether higher protein intakes are superior at maintaining physical performance. Lastly, we aimed to determine whether high protein intakes could mitigate adaptive thermogenesis (AT) in response to ER.

Methods

Twelve college aged athletes (6 women and 6 men) from various sports were analyzed in this study. They underwent a 6-week 25% reduction in energy intake along side a 3-day full body resistance training program. Participants were randomly assigned to a low (~1.2g/kg), moderate (~1.6g/kg) or high (~2.2g/kg) protein group. Baseline and post-intervention measures were obtained via Dual X-ray absorptiometry (DXA), isotopic water dilution, indirect calorimetry, dietary records and strength based physical testing.

Results

Our results indicate a main effect of the intervention on fat mass reductions (19.66 ± 9.05 kg vs 18.02 ± 8.07 kg) (p = 0.016) despite non-significant reductions in body weight (p = 0.059). No significant changes in FFM were observed (p = 0.307). Significant increases in chest press 5RM strength (43.18 ± 14.95 kg vs 48.86 ± 17.46 kg) and chin-up maximal repetition (4.75 ± 5.64 vs 6.08 ± 6.56) were observed across all groups from baseline to post-intervention ($p < 0.05$). No significant increases in strength were noted on lower body movements. Resting energy expenditure (REE) was significantly reduced after the 6-week intervention (1743.52 ± 295.74 kcal vs 1655.18 ± 263.23 kcal) (p = 0.006). What is more, post-intervention measured and predicted REE were also significantly different (1655.18 ± 263.23 kcal vs 1747.92 ± 263.20) (p = 0.012). No significant effects of protein were noted on any of the outcomes.

Conclusion

Our preliminary results indicate that the ACSM recommendation of 1.2-1.7g/kg is sufficient for most athletes even during periods of ER to maintain FFM and physical performance. We also found the possibility of early AT in that population independently of protein intake. In fact, REE was reduced by ~100 kcal/day more than predicted despite minimal weight loss and relative preservation of FFM.

TABLE OF CONTENTS

List of Abbreviations

Abbreviation	Definition
AA	Amino Acids
ACSM	American College of Sport Medicine
AE	Aerobic Exercise
Akt	Protein kinase B
AMPK	AMP-activated protein kinase
ANOVA	Analysis of Variance
BMR	Basal Metabolic Rate
CHO	Carbohydrates
CR	Caloric Restriction
CSA	Cross-Sectional Area
D_2O	Deuterium Oxide
DXA	Dual energy X-ray absorptiometry
EAA	Essential Amino Acids
EE	Energy Expenditure
EI	Energy Intake
ER	Energy Restriction
fCSA	Fiber Cross-Sectional Area
FFM	Fat-Free Mass
IAAO	Indicator Amino Acid Oxidation
ICF	Intracellular Fluid
ILWO	Individuals living with obesity
LBM	Lean Body Mass
mCSA	Muscle Cross-Sectional Area
MM	Muscle Mass
MPB	Muscle Protein Breakdown
MPS	Muscle Protein Synthesis
mTOR	Mechanistic target of rapamycin
N	Nitrogen
NEAA	Non-Essential Amino Acids
NREE	Non-Resting Energy Expenditure
P70S6K	Ribosomal protein S6 kinase beta-1
PAL	Physical Activity Level
RDA	Recommended Daily Allowance
REE	Resting Energy Expenditure
RM	Repetition Maximum
ROS	Reactive Oxygen Species
RT	Resistance Training
TBW	Total Body Water
TDEE	Total Daily Energy Expenditure
TEF	Thermic Effect of Feeding

Introduction

Weight loss practices are common amongst athletes competing in sports where lower body fat levels and higher fat-free mass (FFM) are perceived as advantageous for physical performance (Rankin, 2002; Trexler et al., 2014). Athletes seek changes in body composition primarily through reductions in body fat stores and retention of skeletal muscle mass (MM) to optimize performance and aesthetics (Huovinen et al., 2015a). The premise behind such practices are improved power to mass ratio, which results in improved athletic performance (Huovinen et al., 2015a). However, periods of energy restriction have been associated with losses of FFM and decrements in physical performance (Melin et al., 2019a). Hence, weight loss protocols geared specifically towards the maintenance of FFM might be needed to achieve greater athletic performance after weight loss. This might explain the emerging popularity of high protein diets which have been shown to facilitate weight loss through increased diet induced thermogenesis and possibly favor the maintenance of FFM during periods of energy restriction, at least in individuals with overweight (Halton & Hu, 2004; Longland et al., 2016). However, less is known about the effects of high protein diets in trained individuals, with lower adiposity. Some have proposed that levels of physical activity and total training volume and intensity could drive the need for more protein during periods of energy restriction (Lemon, 2000). In contrast, others believe that leaner individuals have a higher risk of losing FFM during periods of caloric restriction (CR) because they have less body fat to support basic physiological energy demands compared to individuals living with obesity (ILWO) (Elia et al., 1999). This is of concern for athletes trying to make weight for competition or for individuals who practice sports where a lower body weight is perceived as a contributor to better performance. The current protein recommended daily allowance (RDA) for

sedentary individuals is 0.8g/kg per day whilst the current RDA for active individuals, which has been recently updated by the American College of Sports Medicine (ACSM) ranges between 1.2-1.7g/kg per day (Wu, 2016). However, the range for protein intake recommended by the ACSM for active individuals might not be representative of an athlete's absolute needs. For example, a study on runners using the indicator amino acid oxidation (IAAO) method indicated that the RDA for protein in those athletes could be as high as 1.83g/kg per day (Kato et al., 2016). Hence, protein recommendations might be specific to the demands of the athlete's sport and in close relationship with total energy expenditure (EE). Similarly, a 2011 review paper on protein recommendation for athletes proposed that athletes undergoing periods of energy restriction might require up to 1.8-2.7g/kg/day in order to maintain lean body mass (Phillips & Van Loon, 2011). However, it has recently been shown that consuming 1.7g/kg of protein during a 2-week 40% energy restriction (ER) diet provided no advantage to changes in FFM compared to an intake of 0.9g/kg in female athletes (Pearson et al., 2021). Moreover, a recent meta-analysis conducted in 2018 suggests that protein intakes >1.6g/kg/day do not present any advantage and do not yield to further increases in FFM (Morton et al., 2018). However, certain limitations related to that analysis make it difficult to draw clear conclusions regarding protein intake requirements for athletes during periods of energy restriction. Firstly, the analysis excluded weight loss trials. Secondly, the analysis only included studies where at least one group was provided with a protein supplement, which might have led to the exclusion of studies reporting important data. Thirdly, the analysis included studies where training frequency was as low as 2x/week, which might not represent the higher training load often seen in trained or more advanced athletes. Finally, it is important to consider that a large proportion of the literature available on protein intake and the impact it may have on body composition often report changes in lean body mass (LBM) or FFM and not specifically MM.

Hence, more studies that use a combination of dual energy X-ray absorptiometry (DXA) and Deuterium Oxide (D_2O) dilution to derive actual tissue changes are needed. For those reasons, it remains to be clearly established as to whether protein intakes higher than the RDA of 1.2g/kg for active individuals represent added value in terms of changes in muscle mass specifically in the context of energy restriction.

History of Protein Requirements

The debate surrounding protein intake has been ongoing for centuries. It was initially proposed in the 1840's by von Liebig that a daily protein intake of 120g would be sufficient to assure the basic physiological needs of the average man (Liebig, 1841). This value was based on the average protein intake reported by German workers at that time and was later challenged by Chittenden in 1904 (Chittenden, 1904). Two observations were made at the time that challenged von Liebig's views: (1) Adults consuming approximatively 60g of protein daily were healthy and in a state of nitrogen balance and - (2) college athletes at the time who consumed approximatively the same amount of protein – around 60g per day – were able to maintain their levels of physical performance (Chittenden, 1904). During the early 1800's until the early 1900's, many unidentified amino acids (AA) were discovered and experiments were conducted to understand their structural and chemical properties (McCoy et al., 1935; Vickery & Schmidt, 1931). In the period of 1930 to 1955, researchers found that certain AA were indispensable and necessary for optimal growth of laboratory rats (Rose, 1968). In fact, the removal of specific AA from hydrolyzed protein sources led to important nutritive failures such as impaired growth, loss of appetite and death (Rose, 1968). Similar experiments were then conducted on college students at the midst of World War II, in part to understand AA needs in young men susceptible for deployment (Rose, 1968). The authors found

that the omission of certain AA, more specifically the dietary removal of one or a combination of either leucine, valine, isoleucine, methionine, tryptophan, lysine, phenylalanine and threonine led to a sturdy negative nitrogen balance, extreme fatigue, irritability and appetite alterations (Rose, 1968). The work of Rose along with the discovery of the 20 AA throughout the past centuries led to the current classification of AA as essential (EAA) and non-essential (NEAA) (McCoy et al., 1935; Rose, 1968; Vickery & Schmidt, 1931). Throughout the 20th century, further studies were conducted to investigate the relationship between protein intake and nitrogen balance and were later compiled together in a 2003 meta-analysis (Rand et al., 2003). The analysis revealed that protein intakes of 0.65-0.8g/kg were sufficient to satisfy the basic requirements of healthy populations at the 97.5th percentile (Rand et al., 2003). In 2005, the Food and Nutrition Board of the Institute of Medicine published a dietary reference intake document in which the RDA for protein is established at 0.8g/kg (DRI, 2005). However, emerging evidence in the first decade of the 21st century challenged this value suggesting that athletes would require protein intakes > 0.8g/kg depending on the type of activity they take part in (Lemon, 2000). Recently, the ACSM updated its stance on protein recommendations for athletes with the now accepted range of 1.2-1.7g/kg (ACSM, 2011). Despite those changes in protein recommendations for athletes, the debate goes on. In fact, many discrepancies arise from scientific literature regarding protein intake and the physiological effects of consuming too much or too little. Moreover, the data regarding protein intake as it relates to athletes in the context of ER is limited. Those discrepancies will be presented and discussed in the following sections.

Skeletal Muscle Hypertrophy

Skeletal muscle hypertrophy is often defined as the occurrence of increased muscle mass or size. However, this definition is incomplete because changes in various compartments within muscle

such as glycogen stores, muscle cross-sectional area (mCSA), fiber cross-sectional area (fCSA), muscle thickness, intracellular fluid (ICF), sarcoplasmic volume and increases in muscle protein content can all result in increases of muscle mass (Haun et al., 2019). Hence, if positive changes in contractile protein content are responsible for most of the potential improvements in physical performance (i.e. strength, power, speed), then the interpretation of the current body of literature must objectively consider limitations of the different measurement tools used in various studies (DXA, MRI, biopsy, ultrasound etc…). For example, positive changes in FFM could mistakenly be interpreted as changes in muscle protein content but this is not always the case. Similarly, studies on muscle hypertrophy that report positive changes in CSA and muscle thickness do not necessarily indicate that changes in myofibrillar protein content or that the addition of sarcomeres occurred. This is because mCSA and muscle thickness can be acutely affected by changes in ICF, glycogen and muscle water content. In fact, eccentric contractions and muscle damage have been shown to cause an acute inflammatory response associated with increases in muscle oedema (Rodenburg et al., 1994). Thus, changes in muscle water content that can occur over the course of several weeks of hypertrophy training are of important consideration when it comes to the proper interpretation of the data regarding muscle hypertrophy and what is meant by that terminology. Moreover, the methodological differences between studies and the omission of muscle water content measurement could lead to the false assumption that a given protocol or experimental condition led to an increase in muscle protein content when in fact the increase in muscle hypertrophy was the result of higher muscle water content and oedema. Ultimately, changes in muscle protein content are dictated by multiple factors that impact the balance between muscle protein synthesis (MPS) and muscle protein breakdown (MBP) and those factors are not exclusive to resistance exercise (Lim et al., 2022). For the most part, they include the inter-dynamic between

nutritional, hormonal, molecular and cellular activity (Lim et al., 2022). Despite the nuances mentioned above and for logistic purposes, muscle hypertrophy will be considered as an increase in skeletal muscle size throughout the following section.

Training Volume

Training volume is thought to be the primary driver of muscle hypertrophy independently of the exercise modality. Accordingly, muscle hypertrophy can occur as the result of both resistance training (RT) and aerobic exercise (AE) (Konopka & Harber, 2014). It was found that running for 45 minutes at 75% of VO_2 resulted in significant increases in fractional protein synthetic rate in both the soleus and vastus lateralis muscle of endurance trained individuals (Harber et al., 2009). In fact, eccentric contractions have been shown to induce muscle hypertrophy through the signaling of mTOR which is considered a major regulator of cellular growth (Goodman et al., 2011; Liu & Sabatini, 2020). However, it has also been found that increases in myofibrillar protein synthesis only occur in response to resistance training and not endurance exercise (Wilkinson et al., 2008) For example, it has been shown that performing leg extensions for 8 sets of 5 repetitions resulted in greater P70S6K phosphorylation in endurance trained men compared to strength trained individuals (Coffey et al., 2006). However, when strength trained individuals performed a bout of endurance exercise, they had higher AMPK and lower Akt phosphorylation levels than endurance trained individuals (Coffey et al., 2006). It is possible that 8 sets of 5 repetitions were not sufficient to impose an overload stimulus in the strength trained group, hence the reason why those individuals displayed a lower anabolic response ensuing the protocol. Nonetheless, a recent meta-analysis reported that resistance training is superior to aerobic training at inducing hypertrophy, both at the whole muscle and myofiber level (Grgic et al., 2019). Despite those findings, the exact reason why resistance exercise induces a molecular response that is superior to aerobic exercise at

inducing hypertrophy if training volume is the main driver remains to be established. In fact, it would be plausible to assume that for an equated number of contractions and matched for volume, hypertrophy between aerobic and resistance training should be the same. However, this is clearly not the case when looking at the morphology of strength trained athletes compared to endurance athletes. This could be because a certain threshold of training intensity must be attained to induce the activation of type II fibers which have a greater hypertrophy potential than type I fibers (Koopman et al., 2006; Sertel et al., 2011). It is also possible that an upper limit of training volume exists at which point doing more volume induces a higher catabolic response that is not conducive to contractile tissue hypertrophy. In addition, the often reported energy deficiencies in endurance athletes who perceive a lower body mass as being favorable for performance could partially explain the different morphologies of these athletes compared to athletes of different demographics (Melin et al., 2019a). For those reasons, training volume will be discussed in terms of resistance training throughout this review.

There are many accepted ways by which training volume can be calculated. For example, the number of sets times the number of repetitions or the number of sets times the number of repetitions times the load (volume load) are common ways of determining training volume (Scott et al., 2016). Increases in vastus lateralis and elbow flexor CSA have been shown to occur from training in a wide range of training intensities (40%-80%) when equated for volume load (Lasevicius et al., 2018). However, it has recently been proposed that calculating volumes in terms of total weekly working sets per major muscle groups might be a more practical measure. This is because muscle growth does not always equate to its mathematical volume load, but often correlates with the number of weekly sets performed for a given muscle group, independently of the repetition range and the load (Israetel et al., 2020). Accordingly, a recent meta-analysis

concluded that a dose response relationship exists between the weekly number of working sets per major muscle groups and hypertrophy, whereby 10 working sets per major muscle group seems to be the minimal effective dose for inducing muscle hypertrophy (B. J. Schoenfeld, Ogborn, et al., 2017). Of important consideration, the analysis included studies mostly conducted on untrained individuals, who tend to be more responsive to resistance training compared to more advanced trainees (Ahtiainen et al., 2003). Moreover, no upper limit of training volume was determined in the latter analysis. Along those lines, a recent paper by the same group reported that training volumes as high as 45 sets per week for a given muscle group compared to 9 sets per week or 27 sets per week led to greater muscle size and thickness after 8 weeks (B. J. Schoenfeld et al., 2019). Discordances in changes between muscle groups were also noted in the latter, whereby not all muscle groups responded better to higher volumes. For example, the authors indicated that changes in elbow extensor thickness did not significantly differ between groups and noted that some muscle groups might be more responsive to higher or lower volumes depending on various factors such as their fiber type composition. Moreover, there was no dietary intervention in the latter except for self-reported diet recalls. In addition, basic anthropometric measurements were not presented in the paper making it impossible to know if all groups were in a state of energy balance. Despite those findings, caution must be taken when interpretating results from the latter study. Training with such high volumes continuously for each major muscle group might ultimately have detrimental effects and lead to diminishing returns since an inversed U-shape relationship between training volume and hypertrophy has been proposed (Faigenbaum, 2009; Figueiredo et al., 2018). The exact mechanisms by which higher training volumes promote hypertrophy are not fully understood. It has been suggested that higher training volumes amplify the anabolic signaling related to exercise, which in turn could lead to greater muscle hypertrophy over time (Figueiredo

et al., 2018). Ultimately, the principle of progressive overload must be respected for continuous progress to occur. Whether it be through increases in intensity or volume might not matter in the short term for as long as a volume threshold of ~10 weekly sets per major muscle group is met.

Mechanisms of Muscle Hypertrophy

When equated for volume, three mechanisms related to exercise are thought to induce the occurrence muscle hypertrophy. Those are referred to as 1) mechanical tension, 2) muscle damage and 3) metabolic stress (B. J. Schoenfeld, 2010a). Mechanical tension refers to a force measured in Newtons that results in the deformation of a target tissue (Burkholder, 2007). In practical terms, mechanical tension can be defined as resistance training with high loads (≥85% 1RM) performed for a low number of repetitions (1-5) and long rest periods (3-5 min) (Krzysztofik et al., 2019). Training with such high loads has been shown to induce significant gains in muscle hypertrophy in multiple studies. However, the lower volumes associated with this type of loading scheme might not allow for maximal hypertrophy to occur (Kraemer et al., 2002). In contrast, neuromuscular adaptations and increases in maximal strength are more likely to occur as the result of training with such high relative intensities of 1RM (B. J. Schoenfeld, Grgic, et al., 2017). Secondly, exercise induced muscle damage, which refers to an acute localized muscular microtrauma in response to exercise, is thought to be conducive to hypertrophy (B. J. Schoenfeld, 2010b). Despite no objective definition of muscle damage, potential indicators include Z-line streaming, loss of thick myofilaments, disturbed A-band filament arrangement, loss of mitochondria, reduced excitation-contraction coupling, increased blood myoproteins and increased markers of inflammation (Clarkson & Hubal, 2002; McGinley et al., 2009). The inflammatory response resulting from exercise induced muscle damage is thought to be associated with the release of

macrophages that in turn could promote tissue regeneration and compensatory hypertrophy (B. J. Schoenfeld, 2012). Moreover, eccentric contractions might disrupt muscle redox balance whereby reactive oxygen species (ROS) are produced endogenously by muscle or exogenously by the immune system in response to such exercise (McGinley et al., 2009). It has been proposed that ROS, at least at low levels, are essential in the signaling cascade leading to muscle regeneration and force production but they might not be directly related to muscle damage (McGinley et al., 2009; B. J. Schoenfeld, 2012). Moreover, eccentric contractions have been shown to induce an increased expression of MyoD – a muscle regulatory factor – to a significantly greater extent than concentric contractions alone in otherwise healthy individuals (Sabouri, 2022). Myogenic regulatory factors have previously been associated with stretch induced muscle hypertrophy despite myonuclei irradiation (Lowe & Alway, n.d.). Practically, performing resistance exercise in the 8-12 repetition range with loads ≥60% of 1RM is thought to cause important disruption of skeletal muscle homeostasis through the sustainability of cross-bridge formation, which could ultimately potentiate hypertrophy (B. Schoenfeld, 2000). Thirdly, "metabolic stress", which has been defined as training with high-repetition low-load training is thought to be conducive to muscle hypertrophy (B. J. Schoenfeld, 2013). This type of training has been associated with the release of molecules coined as metabolites which primarily include lactate, hydrogen ions, inorganic phosphate, phosphocreatine, ROS, nitric oxide, heat shock proteins and myokines such as Irisin (Lee & Jun, 2019; Lim et al., 2022; B. J. Schoenfeld, 2013). It has previously been proposed that those metabolites could be directly or indirectly be conducive to hypertrophy through the upregulation or downregulation of anabolic and catabolic pathways respectively (Loenneke et al., 2010). Moreover, a low intensity (20% 1RM) bilateral leg extension blood flow restriction protocol has been shown to increase MPS by 46% and 56% in both young and older

men respectively compared to no changes in control groups (Fry et al., 2010; Fujita et al., 2007). Whether those increases in MPS were the result of metabolite accumulation is unclear. A recent review on the topic reported that no causative relationship between metabolite build-up and muscle hypertrophy exist (Lim et al., 2022). Nonetheless, traditional high-repetition low-load training has been shown to induce significant increases in elbow flexors, elbow extensors and quadricep muscle thickness (B. J. Schoenfeld et al., 2015). Those findings suggest that muscle hypertrophy can occur even when training at lower intensities of 1RM independently or not of metabolite accumulation. Hence, performing resistance exercise in the 15-30 repetition range with loads ≤50% of 1RM could be conducive to muscle hypertrophy despite the mechanisms not being completely understood.

Resistance Training & Energy Restriction

The importance of resistance training for the maintenance of MM during periods of energy restriction is well established (Sardeli et al., 2018). However, *how* to train during such periods is less clear and leads us to a metaphoric dilemma. Indeed, if training volume is truly the main driver of muscle hypertrophy, then increasing it during such periods would be of important consideration for the dieting athlete. On the other hand, it would also be plausible to think that the lower energy availability caused by the energy deficit would limit the ability of the athlete to perform more volume with the same relative intensity. According to this line of thought, muscle mass loss during periods of restriction would be inevitable because for a given training volume, it would not be possible to maintain relative intensity and vice-versa. Hence, some might believe that reducing training volumes during such periods would be more beneficial for the maintenance of MM since it would match the energy availability of the athlete allowing for relative intensity to be maintained. However, a recent review reported that higher training volumes (10-30 weekly sets per major muscle groups) or higher training tonnage (reps x sets x load) were associated with greater FMM

retention during periods of CR (Sardeli et al., 2018). However, a few caveats must be considered. Firstly, only 4 of the 15 studies included in the latter reported the data necessary to calculate the weekly sets performed per major muscle groups or the tonnage. Secondly, protein intake and the training routines were reported, but not controlled for by the researchers in any of those 4 studies (Mitchell et al., 2018; Pardue et al., 2017; Petrizzo et al., 2017; Tinsley et al., 2019). Nonetheless, it can be assumed that higher training volumes during periods of increased catabolism would be required to somewhat maintain eucaloric or hypercaloric levels of anabolism. However, to our knowledge, this assumption has not been adequately tested. Moreover, it is unknown whether protein intakes greater than the current RDA for active individuals (>1.2-1.7g/kg) could make up for the reduced anabolic drive caused by a compromised ability to perform high volume training during periods of CR, if this is indeed the case. Those interrogations are to be addressed in more detail in the following sections.

Protein Balance & Energy Restriction

Protein oxidation is thought to be relatively constant during periods of energy balance (Péronnet, 1991; Péronnet & Haman, 2019). In fact, protein oxidation is thought to contribute for 12-15% of total daily energy production independently of exercise or physical activity level (PAL) (Rennie & Tipton, 2000). It has also been shown that consuming more protein than relative needs leads to proportionally higher nitrogen (N) excretion (Price et al., 1994). In fact, a 4-fold increase in daily urea excretion was noted in participants who consumed ~2.3g/kg of protein compared to ~0.77g/kg (Price et al., 1994). However, despite the increases in N excretion, higher N intakes resulted in significantly higher N retention as well. It has also been suggested that exercise does not increase the need for greater protein intakes and that consuming too much protein results in an increased

metabolic efficiency to oxidize protein (Rennie, 1999). In fact, exercise is thought to play an important role in the regulation of protein balance whereby some believe that exercise increases protein utilization efficiency and actually reduces protein requirements in trained individuals (Butterfield & Calloway, 1984; Rennie & Tipton, 2000). However, it is unclear whether the increased N retention efficiency is caused by exercise itself. It could well be caused by the proportional increase in energy intake (EI) resulting from the increased EE related to exercise, at least in weight stable individuals. Nonetheless, it has been previously reported that in a state of energy balance or during periods of energy surplus, consuming more than 1.6g/kg of protein offers no benefits in terms of positive changes in FFM (Morton et al., 2018). On the other hand, periods of energy restriction have been shown to increase N excretion especially in leaner individuals, which precipitates protein requirements during such periods, independently of the training status (Elia et al., 1999). In fact, it has been demonstrated that a negative correlation exists between initial body fat levels and protein contribution to basal metabolic rate (BMR) during a 16-day starvation protocol (Elia, 1992). Hence, higher body fat levels would serve to provide an energy supply sufficient to maintain FFM during periods of CR, at least temporarily. Along those lines, periods of CR are thought to induce the activation of AMPK and other catabolic pathways that could result in mTOR downregulation and negatively impact MPS (McIver et al., 2012). Accordingly, muscle atrophy caused by limb disuse has been shown to be induced by a reduction in MPS rather than an increase in MPB (Phillips et al., 2009). Providing that resistance training and AA are the primary regulators of MPS, it would be plausible to assume that any modulation in one or both these variables could allow for the maintenance of FFM during periods of reduced anabolism such as CR. Indeed, retention of FFM has been shown to occur in a study investigating changes in body composition and MPS during a 21-day 40% energy restriction period (Pasiakos et al., 2013). A

significant increase in the proportion of FM to FFM loss was observed in groups with protein intakes representing 2x-RDA or 3x-RDA compared to an RDA equivalent group (Pasiakos et al., 2013). Interestingly, training volume was relatively low in the latter study (3 sets of 15 repetitions per major muscle group performed 3 times per week). Moreover, the authors indicate that the intensity was also low to prevent a novel stimulus effect on their measured outcomes (Pasiakos et al., 2013). Based on those data, it is unclear if higher training volumes alongside high protein intakes (2-3x RDA) would have yielded even greater changes in FFM. A similar study on young overweight men who were put on a 40% CR diet with a daily protein intake of either 1.2g/kg or 2.4g/kg showed that the higher protein group gained ±1.2 kg of LBM while simultaneously losing ±5 kg of FM compared to the lower protein group who saw no changes in LBM and a reduction of ±3.5 kg in FM (Longland et al., 2016). Of note, training volume was much higher in the latter compared to the study conducted by Pasiakos. However, most of the training volume came from high glycolytic anaerobic conditioning (i.e wingates, sprints, circuits) rather than traditional RT with high mechanical tension. As mentioned in the previous section, "metabolic stress", which potentially occurs during those types of activities, might not be the most conducive to muscle hypertrophy (Lim et al., 2022). Nonetheless, those studies provide solid evidence that high protein intakes are beneficial for FFM retention during periods of ER. However, certain limitations are to be considered before definitive conclusions can be drawn. Firstly, both studies conducted an energy restriction protocol that lasted only 21 and 28 days respectively (Pasiakos et, al. 2013; Longland et al., 2016). Those dieting patterns might not reflect those used by athletes in various sports who tend to restrict calories for longer periods (Melin et al., 2019b). Secondly, and as previously mentioned, changes in FFM do not necessarily reflect changes in actual MM. Hence, definitive conclusions regarding changes in MM based on those studies cannot be made (Pasiakos

et, al. 2013; Longland et al., 2016). Thirdly, participants in Longland's study were untrained which could have affected the outcome of the results (Longland et al., 2016). Finally, there is evidence suggesting that substituting carbohydrates (CHO) for more protein might ultimately be detrimental for MM retention since the MPS response from resistance training is compromised when one is subjected to train in a glycogen depleted state (Tipton, 2011). For those reasons, it is unclear whether protein intakes >1.6g/kg during periods of CR offer a true benefit in terms of MM retention.

Protein, Weight Loss & Physical Performance

The sole purpose of losing weight as it relates to athletic performance is to improve power to mass ratio which features lower body fat levels and higher MM (Murphy et al., 2015). However, some studies indicate that a lower body weight and lower body fat levels do not necessarily equate to better athletic performance (Melin et al., 2019b). Moreover, little data is available regarding the effects of high protein diets on exercise performance during periods of ER. It has previously been demonstrated that a protein intake of 3g/kg led to an acute reduction in muscle soreness 24h following an eccentric exercise protocol compared to an intake of 2g/kg (Shamim et al., 2018). However, there was no difference in muscle soreness nor muscle function between groups after 72h making it difficult to determine whether extremely high protein intakes are truly relevant for muscle recovery. Moreover, an 8-week study on female physique competitors investigated the effects of a high protein (2.4g/kg) diet compared to a low protein (1.2g/kg) diet on body composition and maximal strength and found significant increases in FFM as well as increases in the squat and deadlift 1RM from baseline in both groups. However, there tended to be greater increases in the high protein group in all the above variables compared to the low protein group

(Campbell et al., 2018). It is important to note that total calories were not controlled for in that study and that the high protein group tended to consume significantly more calories on average compared to the low protein group which possibly influenced the outcome. Moreover, the study was not specifically designed to induce ER and its effect on performance. On the other hand, a cross-over study on weightlifters undergoing a 2-week 40% CR diet with protein intakes of either 1.6g/kg or 2.8g/kg and matched for CHO intake found that higher protein resulted in subjectively better mood but was slightly inferior at maintaining strength (Helms et al., 2015). There is also mixed evidence regarding weight loss itself and its impact on exercise performance independently of protein intake. For example, a study on swimmers reported a 9.8% decrease on 400-m swim velocity related to energy deficiency throughout a 12-week competitive season (VanHeest et, al. 2014). Along those lines, a study on middle aged non-obese men found that a 10-week 20% ER protocol led to significant decreases in maximal cycling power output but had no impact on submaximal exercise performance (JMVelthuis-te Wierik, E et al., 1994). Similarly, results from a bodybuilding contest preparation case study indicated a 17% decrease in vertical jump height after the intervention but showed an actual increase in maximal isometric knee extension strength (B. J. Schoenfeld et al., 2020). Thus, it is possible that activities requiring higher neuromuscular demands such as maximal speed and velocity might be more sensitive to energy restriction than strength and hypertrophy. In addition, changes in physical performance that occur throughout a weight loss phase might be population specific. In fact, a study on overweight men found increases in maximal strength on the bench press and the leg press following a 4-week 40% caloric restriction protocol (Longland et al., 2016). In contrast, a review on weight-cutting in strength sports such as powerlifting and Olympic weightlifting indicates that weight loss >2-3% of initial bodyweight could negatively affect performance in those athletes (Moore et al., 2019). Those results indicate

that trained individuals are more at risk of seeing performance decrements during weight loss phases compared to untrained individuals. In addition, there is evidence suggesting that the rate of weight loss might dictate performance outcomes. In fact, it has been shown that a weekly rate of weight loss equivalent to 0.7% of initial bodyweight was better than a rate of 1.4% at maintaining and even increasing maximal strength, power and LBM (Garthe et al., 2011). It is plausible to assume that rapid weight-cutting used by powerlifters and Olympic weightlifters might lead to performance decrements that could be attenuated with a protocol inducing slower rates of weight loss. In addition, rapid weight loss has been associated with impairments in muscular endurance as well as aerobic and anerobic capacities which could be detrimental to athletes practicing sports such as rowing or combat (Sundgot-Borgen & Garthe, 2011). Finally, low glycogen levels have been associated with impaired performance during high-intensity activities (Vigh-Larsen et al., 2021). Thus, it is possible that substituting CHO for more protein during periods of CR might have detrimental effects on physical performance.

Seemingly, periods of CR do not provide a favorable physiological environment that is conducive to improvements in physical performance. However, based on current evidence, it is difficult to determine whether higher daily protein intakes (>1.6g/kg) could help mitigate the possible performance decrements that might occur during periods of ER. Moreover, it seems like maintenance or improvement in training performance during hypocaloric periods could be affected by multiple factors such as the rate of weight loss, the magnitude of weight loss, the training status of the individual, initial body fat levels and macronutrient distribution.

Conclusion

Athletes who undergo periods of energy restriction seek changes in body composition that are conducive to improved physical performance. This is achieved through an increased power to mass ratio which results from the loss of fat-mass and the preservation of FFM. However, leaner individuals tend to have greater N excretion levels than ILWO during periods CR, which could result in the net loss of MM (Elia et al., 1999). It has previously been shown that protein intakes greater than the current RDA alongside resistance training could prevent the loss of FFM during periods of CR (Pasiakos et, al. 2013; Longland et al., 2016). However, mixed evidence exist in those regards with recent data suggesting no benefit of increased protein in female athletes during periods of CR (Pearson et al., 2021). Moreover, the interpretation of the current body of literature must account for the lack of direct evidence for changes in actual muscle tissue mass rather than changes in FFM as reported in many studies. For those reasons, it remains uncertain as to how much protein is necessary to maintain MM during periods of CR, if this is indeed possible.

Research Question

The purpose of this study was 3-fold. The primary objective was to determine whether high protein intakes could maximise fat-free mass retention in normal weight trained individuals during periods of energy restriction. The secondary aim was to determine if a higher protein intake is superior at maintaining physical performance during periods of ER. Finally, the third aim was to determine if higher protein intakes could attenuate the physiological response to weight loss such as greater than predicted decreases in REE.

Hypotheses

We hypothesized that higher protein intakes (>1.6g/kg) would yield greater retention of FFM. We also hypothesized that higher protein intakes (>1.6g/kg) would be associated with greater retention of exercise performance. Lastly, it was also hypothesized that lower protein intakes (<1.6g/kg) would be associated with greater reductions in REE compared to higher protein intakes.

Materials & Methods

Population

Fourteen participants (8 men, 6 women) aged 22.5±4.1 years were included in the study. Two participants dropped out of the study after the pre-intervention tests. The initial screening process included both recreational and competitive drug-free athletes who had been resistance training for the past three months or more prior to their enrollment in the study. Individuals with an underlying health condition that could have been aggravated by the experimental protocol were not considered for this project. In addition, participants who had purposefully lost weight or sustained a caloric deficit in the 6 months leading to the experiment were excluded (n=1). Finally, athletes who were

to be in-season at the time of trial or who could not engage in a 3-day resistance training protocol during the trial were excluded (n=2).

Study Design

We conducted a 6-week randomized control trial where participants were randomly assigned to 1 of the 3 following groups: (1) Low protein (LP ~ 1.2g/kg), (2) Moderate protein (MP ~ 1.6g/kg) or (3) High protein (HP ~ 2.2g/kg). A 25% caloric deficit was applied to participants of all 3 groups for the entire duration of the study. Participants were allowed to maintain their regular practice/training schedule throughout the entire 6-week intervention.

Testing & Measurements

Participants were required to visit the laboratory on 4 occasions. All visits occurred at the Behavioural & Metabolic Research Unit (BMRU) at the University of Ottawa. Testing sessions occurred twice before and twice after the 6-week intervention was completed. Participants were instructed to visit the laboratory for testing after an overnight fast. They were also instructed to refrain from vigorous exercise 24h before testing. The experimental sessions were separated into 2 sessions of ~180 and ~75 minutes respectively with a washout period of at least 24h. The sessions were repeated before and after the 6-week intervention.

Experimental Session #1

Deuterium Oxide Dilution

Deuterium oxide dilution was used as a technique to estimate total body water (TBW) of our participants. Upon arrival at the laboratory, participants were asked to provide a sample of saliva by spitting in a collect jar. Samples of 0.6-1.2 ml of saliva were collected and transferred to 12 ml

borosilicate glass vials (Isomass) and stored in a 5ºC standardized disposal fridge. Participants were then provided with 11g of filtered 99.8% D2O (Sigma-Aldrich) along with 150 ml of tap water and asked to drink the solution as previously described (Moon et al., 2008). A period of 180 min was allowed for the enrichment of body water to reach equilibrium before saliva samples were again collected (IAEA, 2010). All samples will be analyzed at the Stable Isotope Laboratory at the University of Ottawa. Covid-19 related delays occurred at the Stable Isotope Laboratory hence, the samples could not be analyzed prior to the deposition date limit of this thesis (see General Discussion, p.37).

Anthropometrics & Body Composition

Height (HR-100 Height Rod; Tanita Corporation of America Inc. Arlington Heights, IL) and weight (HR-100; BWB-800AS, Tanita Corporation, Arlington Heights, IL., USA) were measured after the dilution protocol and prior to the body composition test. Fat mass and FFM were measured by dual energy X-ray absorptiometry (General Electric Lunar Corporation version 6.10.019, Madison, USA). Coefficient of variation and correlation for the dual-energy X-ray absorptiometry was 1.8% (R.0.99) as determined in 12 healthy subjects. All the measurements occurred in the morning after an overnight fast. Participants were asked to wear light clothing (sweatpants, t-shirt, shorts or leggings) and to remove all jewellery (bracelets, necklaces, rings) prior to all measurements.

Indirect Calorimetry

Resting EE was measured by indirect calorimetry (SensorMedics Delta Track II, Datex-Ohmeda, Helsinki, Finland) using the open-hood ventilated technique as previously described (Hintze et al., 2018). Briefly, concentrations of CO_2 and O_2 were measured for 30 minutes. The first 5 and last 5

minutes were omitted from our calculations and 24h REE was derived from the remaining 20 minutes using Weir's equation. Participants were asked to lay down on their back on a hospital bed and remain as relaxed as possible without falling asleep. The test occurred in a quiet room standardized at a temperature of 22ºC. All measurements occurred between 8:00 and 11:00 am after an overnight fast. Ethanol burning tests are routinely performed to control the quality of measurements, as previously described (Cooper et al., 2009; Blond et al., 2011). Our most recent analyses demonstrated -5% and 4.76% difference in CO2 and O2 measures, respectively.

Experimental Session #2

Physical Testing

All physical testing occurred in the training facility of the BMRU laboratory at the University of Ottawa. For logistic reasons, participants were asked to perform the physical tests in the morning after an overnight fast. The tests were performed in the same order for all participants and included:

1) Broad jump (average of 3)
2) Chin-up (1 attempt, max repetition, unloaded)
3) Bilateral chest press (5RM)
4) Unilateral leg press (5RM)
5) Bilateral leg curl (5RM)

The tests were performed after a 5–10-minute warm-up on a treadmill or stationary bike. Participants were instructed to perform each test with maximal intent and to try and reach the highest possible scores. A warm-up set was performed prior to all tests. Strength tests began with a load perceived comfortable by the participants. Rest periods of up to 180 sec were offered between tries and between tests. The loads were gradually increased for the 5RM tests by a member

of the research team until failure to complete 5 repetitions was reached (Hintze et al., 2018; Lasevicius et al., 2018).

Nutritional Intervention

Participants were asked to wear an accelerometer (SenseWear Pro 3 Armbands©, HealthWear Bodymedia, Pittsburgh, PA, USA) for at least 3 days after the first experimental testing session. A 3-day average was used as a proxy of daily non-resting energy expenditure (NREE). A 25% energy restrictive meal plan was designed specifically for every participant based on their total daily energy expenditure (TDEE) and the protein group they were randomized to. Participants were blinded to the group they were designated to. TDEE was calculated from the sum of REE, NREE and thermic effect of feeding (TEF), which was assumed to represent 10% of energy requirements (Calcagno et al., 2019). NREE was extracted from accelerometry and calculated as a 3-day average. TEF, TDEE and the prescribed energy intake (EI) for all participants were calculated using the following equations:

1) $TDEE =$ (REE + NREE) * TEF
2) $EI = TDEE * 0.75$

Participants received a personalized nutritional template based on their EI requirements and group designation as previously described (Hintze et al., 2019) . Briefly, our templates were based on the food exchange system from the Canadian Diabetes Association. This system categorizes foods into 7 groups (dairy, meat & substitutes, fats, bread & whole grains, fruits, vegetables and condiments including tea, coffee and sugar free beverages like diet soda. Participants were prescribed a specific number of items from each food group based on their individual needs.

Resistance Training Intervention

A standardized 3-day full body resistance training program was designed by the research team and explained to all participants at the end of the 2nd experimental session. The program was designed according to recent findings on muscle hypertrophy research as previously described (B. Schoenfeld et al., 2019; B. J. Schoenfeld, 2010a; B. J. Schoenfeld, Ogborn, et al., 2017). Briefly, the program included at least 10 working sets per major muscle groups and included a repetition range of 6-25. An increase in set volume was implemented on week 4 of the program to ensure progressive overload. In addition, participants were asked to progressively increase the loads used on all exercises from week to week whenever possible. Participants were also instructed to perform every set of every exercise within 1-2 repetitions of concentric failure. A sample of the program is presented in the appendices.

Statistical Analysis

Descriptive statistics and one-way multivariate analysis of variance (ANOVA) were used to analyze the baseline characteristics of our participants. All our outcome variables including FFM, FM, TBW (TBD), REE and physical performance were analyzed using ANOVA repeated measures for both within-subjects factors and between-subjects with a protein factor. In case of a significant interaction, post hoc pairwise analyses were conducted using Tukey's adjustments for multiple comparisons. The p-value for statistical significance was set at 0.05. Linear regression was used to predict changes in REE from baseline to post-intervention using FFM and FM as the main predictors. All statistical analyses were conducted with JASP 0.14.1.

Results

Body Composition & Resting Energy Expenditure

There was an effect of the intervention on the loss of FM across all groups (19.66 ± 9.05 kg vs 18.02 ± 8.07 kg) (p = 0.016) despite non-significant reductions in body weight (p = 0.059). No significant changes in FFM were observed (p = 0.307). A significant decrease in REE was observed across groups (1743.52 ± 295.74 kcal vs 1655.18 ± 263.23 kcal) (p = 0.006) between baseline and post-intervention measurements. In addition, REE decreased to a greater extent than predicted by our linear regression model using FM and FFM as the main predictors (1655.18 ± 263.23 kcal vs 1747.92 ± 263.20) (p = 0.012, R^2 = 0.863) (**Figure 1.**). No effect of protein intake was noted for any of the described outcomes (**Table 2**).

Table 1. Participant's characteristics before the intervention[1].

	LP	**MP**	**HP**	***p***
Age	20.5±1.7	23.55±5.0	21.4±1.2	0.403
Body Weight, kg	77.1±13.19	75.0±21.8	65.2±6.1	0.521
Height, cm	172.1±2.6	169.9±8.6	157.9±7.9	0.036*
BMI	26.0±3.9	25.6±4.7	26.4±4.3	0.964
Fat Mass, kg	21.5±9.1	19.62±11.8	17.8±8.3	0.867
Body Fat %	27.2±8.6	25.1±4.7	26.9±10.7	0.945
FFM, kg	52.7±6.7	52.8±12.6	45.1±6.1	0.411

[1]Values are means ± SD's. n = 12 (4/group).
* Significant height difference between LP and HP.
p values were obtained from a one-way ANOVA.

Table 2. Participant's anthropometric and physical performance variables before and after the intervention[1].

	LP		MP		HP		*p*	*p*	*p*
	Pre	Post	Pre	Post	Pre	Post	WS	TP	BS
Body Weight, kg	77.1±13.19	74.8±12.1	75.0±21.8	72.5±19.0	65.2±6.1	66.0±6.7	0.059	0.110	0.580
Fat Mass, kg	21.5±9.1	18.4±9.3	19.62±11.8	18.0±9.2	17.8±8.3	17.7±8.2	0.016*	0.138	0.944
Fat-Free Mass, kg	52.7±6.7	53.6±6.7	52.8±12.6	52.1±11.8	45.1±6.1	46.0±7.2	0.307	0.106	0.443
Broad Jump, cm	201.8±31.1	199.3±34.3	200.2±35.8	189.2±35.6	183.3±35.2	179.9±55.3	0.604	0.997	0.787
Chin-up, max rep	6.5±5.1	8.5±6.5	3.3±5.9	3.8±6.8	4.5±7.0	6.0±7.4	0.003*	0.228	0.691
Chest Press, 5RM, kg	47.7±15.5	55.7±17.5	43.2±17.2	48.9±20.5	38.6±15.1	42.0±16.3	0.00013*	0.176	0.655
Unilateral Leg Press Right, 5RM, kg	47.7±10.8	53.4±13.1	52.8±15.5	50.0±14.9	44.3±6.8	51.1±7.8	0.462	0.347	0.728
Unilateral Leg Press Left, 5RM, kg	45.5±9.8	52.3±13.6	51.1±15.0	48.9±16.3	44.3±13.6	53.4±10.1	0.316	0.816	0.992
Leg Curl, 5RM, kg	39.8±10.1	45.5±9.8	35.2±12.5	40.9±11.7	29.5±7.9	33.0±8.6	0.747	0.505	0.153

[1]Values are means ± SD's. n = 12 (4/group).
p values were obtained from repeated measures ANOVA
WS = Within Subjects. TP = Time and Protein interaction. BS = Between Subjects.
*Significantly different from pre-intervention within subjects (p <. 0.05).

Physical Performance

Substantial increases in chest press 5RM strength (43.18 ± 14.95 kg vs 48.86 ± 17.46 kg) and chin-up maximal repetition (4.75 ± 5.64 vs 6.08 ± 6.56) were observed across all groups from baseline to post-intervention ($p < 0.05$). Significant increases in relative 5RM chest press strength (strength to mass ratio) were also observed across all groups (0.6 ± 0.185 vs 0.686 ± 0.213) ($p < 0.05$). No differences between groups on changes in chest press relative strength from pre- to post-intervention were found ($p = 0.09$).

No significant increases in leg curl 5RM and leg press 5RM were found. No significant changes in broad jump distance occurred (**Table 2**). No significant differences were found in relative 5RM

strength on any lower body movement. We found no differences in relative strength change from pre- to post-intervention between groups for lower body movements.

No effect of protein intake was noted on any of the physical performance parameters that we assessed. Moreover, no significant differences between groups were observed on physical performance changes from pre- to post-intervention on any of our tests.

Table 3. Oxygen consumption, carbon dioxide production, respiratory exchange ratio and resting energy expenditure before and after the intervention[1].

	LP		**MP**		**HP**		***p***	***p***	***p***
	Pre	Post	Pre	Post	Pre	Post	WS	TP	BS
VO_2, L/min	0.258±0.031	0.233±0.039	0.293±0.038	0.263±0.050	0.230±0.027	0.225±0.013	0.008*	0.219	0.181
VCO_2, L/min	0.225±0.026	0.193±0.046	0.250±0.036	0.217±0.045	0.193±0.022	0.188±0.025	0.002*	0.093	0.280
RER	0.881±0.041	0.817±0.039	0.851±0.068	0.831±0.014	0.851±0.071	0.830±0.077	0.210	0.720	0.941
REE, kcal	1803.1±196.8	1615.4±303.3	1847.9±439.4	1825.4±363.2	1579.5±183.8	1567.3±98.2	0.006*	0.102	0.186

[1]Values are means ± SD's. $n = 11$.
p values were obtained from repeated measures ANOVA
WS = Within Subjects. TP = Time and Protein interaction. BS = Between Subjects.
*Significantly different from pre-intervention within subjects ($p <. 0.05$).

Table 4. Energy requirements and macronutrient distribution across all three groups[1].

	LP		**MP**		**HP**	
	Prescribed	Consumed	Prescribed	Consumed	Prescribed	Consumed
EI	1954.30±29.87	1776.08±157.24	2260.1±150.8	2226.08±101.68	2076.02±411.99	1955.50±430.62
Protein g/d	108.33±14.50	100.75±13.43	118.5±17.68	118.5±17.68	145±0	148.5±9.19
CHO g/d	231.33±7.77	207.25±28.66	271±9.9	263.5±0.70	200±79.20	178.25±100.76
Fat g/d	66.67±3.06	60.89±2.77	77±2.83	77.61±3.69	73.8±9.90	72.06±7.15
Protein g/kg	1.3±0.1	1.2±0.1	1.69±0.01*	1.69±0.01*	2.27±0.04**	2.32±0.18**
CHO g/kg	2.9±0.4	2.6±0.6	3.9±0.5	3.81±0.62	3.12±1.19	2.78±1.53
Fat g/kg	0.83±0.13	0.76±0.12	1.11±0.14	1.12±0.13	1.22±0.14	1.13±0.1
Protein %	22.15±2.63	22.70±2.52	20.91±1.73	21.24±2.21	28.50±5.66	31.34±8.78
CHO %	47.36±1.88	46.56±2.75	48±1.45	47.4±2.29	37.76±7.76	35.04±12.89
Fat %	30.72±1.77	30.94±1.53	30.68±0.92	31.38±0.1	31.55±3.16	33.62±4.11
Compliance %		90.7±8.1		98.2±2.6		95.6±2.2

[1]Values are means ± SD's. $n = 7$.
* = Significantly different from LP ($p <. 0.05$).
** = Significantly different from MP ($p <. 0.05$).

Discussion

We conducted a randomized controlled trial looking at the effects of varying protein intakes on body composition, physical performance parameters and REE during a 6-week 25% ER protocol on athletes from various sports. Participants were randomized and assigned to either a LP group (~1.2g/kg), a MP group (~1.6g/kg) or a HP group (~2.2g/kg). We also included a 3-day RT program predominantly geared towards muscle hypertrophy. The program was standardized for weekly set volume as previously suggested and utilized by others (Israetel et al., 2020). The main findings of this study indicate that there were no significant advantages to higher protein consumption on either fat loss or FFM retention (**Table 2**.). Our results also indicate that athletes can maintain their relative physical performance during periods of moderate ER. We found no effect of protein intake on any physical performance parameter. In addition, we found that athletes experience greater than predicted decreases in REE despite minimal WL.

Our results are in line with those obtained from a recent study conducted on female athletes undergoing a 2-week 40% ER protocol with protein intakes of either 0.9g/kg or 1.7g/kg (Pearson et al., 2021). Average weight loss in both groups was ~1.1 kg with no differences in FM and FFM changes between groups (Pearson et al., 2021). A similar study by the same group also reported no differences in the loss of FM in resistance trained men consuming either 1.0g/kg or 2.3g/kg of protein during a 2-week 40% ER protocol (Mettler et al., 2010). However, the low protein group lost significant amounts of LBM compared to no changes in the high protein group. On the other hand, many have suggested that protein intakes ≥1.6g/kg during periods of ER would be more conducive to favorable changes in body composition than lower intakes (Lemon, 2000; Murphy et al., 2015; Phillips & Van Loon, 2011; Tipton, 2011). The reason for those discrepancies might ultimately be due to methodological differences between studies as well as the targeted population.

For example, our study included both male and female athletes from various sports, which was not the case in most studies discussed earlier (Pasiakos et al., 2013 ; Longland et al., 2016; Mettler et al., 2010; Pearson et al., 2021). In addition, our study included a structured RT program geared specifically towards promoting muscle hypertrophy. Other studies also included an exercise intervention however, the intervention was mostly geared towards promoting fat loss rather than persevering FFM (Pasiakos et al., 2013; Longland et al., 2016). Hence, despite our data showing no advantage to higher protein intakes, we cannot disregard the possibility that protein intakes ≥2.2g/kg could exert favorable outcomes in terms of body composition in different populations or in conjunction with different exercise modalities. Moreover, our HP group was the only group that included more females than males indicating that there could be an effect of sex on the responsiveness to protein intake, but we cannot draw clear conclusions with our current sample size.

Another important finding in the current study was the maintenance of physical performance. As previously mentioned, athletes seeking to lose weight do so ultimately to improve their power to mass ratio and relative strength (Huovinen et al., 2015). We found significant increases in chest press 5RM strength (43.18 ± 14.95 kg vs 48.86 ± 17.46 kg) and chin-up maximal repetition (4.75 ± 5.64 vs 6.08 ± 6.56) ($p < 0.05$). The increase in relative strength from pre- to post-intervention on the chest press was also significant (0.6 ± 0.185 vs 0.686 ± 0.213) ($p < 0.05$). However, no changes occurred in 5RM leg curl strength, 5RM leg press strength and broad jump distance. The reason for the preferential strength increases in upper body movements compared to lower body movements is unclear. It could be that our participants came primarily from lower body dominant sports (cycling, football, ice hockey, ringette and soccer) and could have been more responsive to upper body exercises (**Table 2.**). Hence, our results indicate that physical performance can be

maintained and improved during periods of ER in trained athletes. Those findings are in agreement with a study looking at different rates of WL on physical performance and body composition in Olympic athletes (Garthe et al., 2011). In the latter, the authors indicate that the 'slow rate weight loss' group significantly improved physical performance whilst no significant changes occurred in the 'fast rate weight loss' group (Garthe et al., 2011). Similarly, a study by Longland and colleagues reported significant improvements 1RM strength on multiple exercises independently of low (1.2g/kg) or high (2.4g/kg) protein intake following a 28d 40% ER protocol (Longland et al., 2016). However, the authors reported significantly greater increases in LBM and significantly greater losses of FM in the high protein groups (2.4g/kg) which was not the case in our study. Based on the results of the latter study and the results of our current study, it does not seem likely that changes in FFM that occur during periods of ER, whether they be positive or negative, are directly predictive of physical performance outcomes (Garthe et al., 2011; Longland et al., 2016). In fact, physical performance was retained across all our groups independently of changes in FFM and protein intake. The reason for this could be the important contribution of the nervous system in activities requiring a high degree of force production such as a 5RM test (Marshall et al., 2011). If this is indeed the case, it would indicate that protein intake during periods of ER is a poor predictor of physical performance outcomes for as long as the minimal requirements are met (~1.2g/kg). This assumption is in line with a recent study on female athletes undergoing a 2-week 40% ER protocol with protein intakes of ~0.9g/kg for the low group and ~1.7g/kg for the high group (Pearson et al., 2021). Indeed, the authors reported no significant differences in physical performance between groups after the protocol except for concentric isokinetic leg extension strength, which favored the high protein group (Pearson et al., 2021). Hence, our hypothesis regarding better performance and FFM retention with higher protein intake is not supported by our

results. Based on our current findings, it seems like the current ACSM recommendations of 1.2-1.7g/kg is sufficient for athletes who wish to maintain their strength during periods of ER.

Our data also suggest that athletes experience greater than predicted decreases in REE for minimal weight loss and relative preservation of FFM. Our linear regression model suggests that REE should have remained similar from baseline to post-intervention when using FM and FFM as the main predictors. However, REE decreased from 1744 kcal/day to 1655 kcal/day ($p = 0.006$) whilst our predicted post-intervention value was 1748 kcal/day. No significant effects of protein intake were observed on changes in REE. In addition, reductions in REE occurred despite an average weight loss of only ~1.3 kg (72.41 ± 14.73 kg vs 71.08 ± 12.9 kg), which was not significant ($p = 0.059$). Hence, since FFM was relatively maintained across our groups, it is possible that changes in FM were primarily responsible for the decrease in REE (19.66 ± 9.05 kg vs 18.02 ± 8.07 kg) ($p = 0.016$). This would concord with the assumption that fat store depletion is one of the main signal in EE regulation (Dulloo & Jacquet, 1998). In fact, it has previously been shown that greater than predicted reductions in REE occur despite non-significant changes in FFM, at least in ILWO (Doucet et al., 2001). Those findings contradict our hypothesis regarding changes in REE whereby it was assumed that the maintenance of FFM could play a primary role in mitigating the adaptive response to ER. In fact, FFM has been shown to be a greater predictor of REE than FM (Nelson et al., 1992). It has previously been suggested that reductions in REE ensuing periods of ER could be in part caused by increased mitochondrial efficiency and decreased expression of uncoupling proteins (Pileggi et al., 2022; Trexler et al., 2014). This could potentially explain the occurrence of greater than predicted reductions in REE in our cohort independently of reductions in FFM as previously observed in lean individuals (Weyer et al., 2000). However, this was not thoroughly investigated in our study. It is also possible that reductions in REE occur more rapidly in

individuals with a high baseline TDEE as a protection mechanism to preserve energy, but we cannot confirm this with our current data. Lastly, the reductions in REE could potentially explain the low occurrence of WL seen in our study.

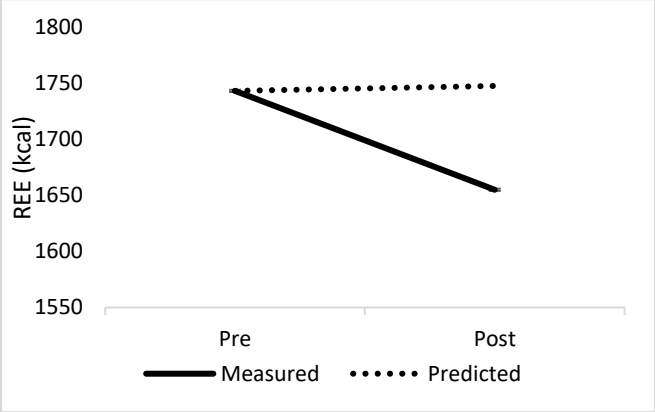

Figure 1. Measured compared to predicted changes in resting energy expenditure (REE) in athletes ensuing a 6-week 25% energy restriction diet with varying protein levels. Measured REE prior to the intervention was 1743.52 ± 295.74 kcal whilst the measured post-intervention REE was 1655 ± 263.23 kcal (p = 0.006). Post-intervention predicted REE was 1747.92 ± 263.20 kcal based on our linear regression model when using fat mass and fat-free mass as the main predictors (REE = (190.925) + (3.408*FM) + (29.582*FFM) (R^2 = 0.863)). A significant difference was observed between post-intervention measured REE and post-intervention predicted REE (1655 ± 263.23 vs 1747.92 ± 263.20 kcal) (p = 0.012).

In fact, it has recently been shown that 1 week of ER can lead to ~100 kcal reduction in 24hEE over what would be expected based on changes in active metabolic tissue (Heinitz et al., 2020). Moreover, reductions in EE on week 1of the protocol was associated with a reduction in total weight loss of ~2 kg on the last week of the protocol (Heinitz et al., 2020). Hence, the possibility of early reductions in REE in our study explaining the low occurrence of WL is conceivable.

Lastly, certain limitations are to be considered with our current study. First, our sample size consisted of only 12 participants. Second, the dietary intervention consisted of a template with equivalents for each food group to be consumed daily. This implies a greater margin of error in terms of calorie and macronutrient consumption compared to a study with pre-made meals given

to each participant for the duration of the study. However, according to our data, compliance to the nutritional intervention across all groups was on average 94.8±3.8% (n=7) with significant differences in relative protein intakes (**Table 3.**). Third, participants were athletes from various sports with different levels of commitment. This allowed for the preservation of the ecological value but was also a limitation because some participants were more active than others. This potentially resulted in some individuals doing more volume of activity than others which could be a confounding factor. Four, protein oxidation was not measured in the current study. This would have provided important information regarding fuel selection with varying protein intakes. Five, this study was conducted during the Covid-19 pandemic resulting in multiple interruptions of normal laboratory operations. This made it difficult to host all our participants for regular training sessions. Thus, the lack of direct training supervision can be considered a limitation as well. However, we observed no significant differences between groups in terms of changes in physical performance from pre- to post-intervention. This indicates that compliance to the RT program was similar between groups. Lastly, delays occurred in other laboratories with whom we work in collaboration. For this reason, we were not able to analyse our D_2O enriched saliva samples prior to the official deposition for this thesis.

Conclusion

In summary, we demonstrate that trained athletes can maintain FFM and improve physical performance during a 6-week 25% ER protocol. We found no advantage nor disadvantage in consuming more protein (~2.2g/kg) on any of our measured outcomes. Hence, we suggest that the ACSM recommendation of 1.2-1.7g/kg is sufficient for most athletes even during periods of ER if a proper RT regimen is implemented. Our data also suggest the possible occurrence of early AT in that population. We found that REE decreased by ~100 kcal more than what was predicted by

our model based on levels FM and FMM. Whether AT subsides once energy balance is re-established will require further investigation.

General Discussion

Motives & General Conclusion

The main purpose of this thesis was to objectively determine if high protein intakes (~2.2g/kg) offer any advantages compared to lower intakes (≤1.6g/kg) on the retention of FFM, the loss of FM and the maintenance of physical performance in athletes during periods of ER. There are three specific motives that led to the development of this project. The first motive came from my own personal experience as an athlete. Over the years, I have purposefully lost weight on multiple occasions as an attempt to improve my own athletic performance for competitive and recreational reasons. Between the ages of 19 and 27, I have tried many different weight loss protocols such as low carbohydrate diets, carbohydrate backloading, carbohydrate cycling, intermittent fasting, extremely high protein diets (>300g/d) and time restricted feeding, each providing their own benefits and drawbacks. Throughout the years, I looked to find the best and most efficient way to lose weight without sacrificing muscle mass and performance. Protein became of particular interest to me because of the way it was marketed in mainstream media. Many of my fellow training partners, teammates and myself believed that since muscle is protein, then more protein must equal more muscle. Moreover, high protein diets used to be marketed as a 'fat burning secret weapon' by supplement companies and fitness gurus. It is needless to say that I developed a bias towards high protein diets! However, that bias slowly faded once I started my bachelor's degree and slowly acquired more and more knowledge about physiology and biochemistry. However, some of my interrogations remained unanswered. This brings up the second motive that led to the development

of this project; anecdotal evidence. Many athletes that I know personally and myself included, have had tremendous success in terms of fat loss and physical performance enhancement eating relatively high amounts of protein (>2.2g/kg). Why is that? Our dietary habits were contrary to the scientific recommendations of 0.8g/kg, later updated to 1.2-1.7g/kg for physically active individuals. Hence, I deducted that there was a mismatch between the scientific evidence and what was being done in practice. I also realized that there were discrepancies within the scientific literature itself regarding protein intake recommendations. Those discrepancies were my final motive for the development of this project.

Many studies have been conducted on protein intake during periods ER however, very few have been conducted on athletes. It was assumed that leaner individuals are more likely to lose FFM during periods of ER because of an increased reliance on protein catabolism for ATP production compared to individuals with more body fat. During periods of energy balance, 1.6g/kg has been shown to maximize gains in LBM in individuals who resistance train (Morton et al., 2018). However, it was unknown whether 1.6g/kg was sufficient to provide the same effect during periods of ER. In addition, the current ACSM recommendation for active individuals (1.2-1.7g/kg) does not necessarily consider training status nor energy status. Hence, it was plausible to assume that higher protein intakes (>1.6g/kg) would be beneficial for athletes during periods of ER. Interestingly, we found no effect of protein intake on any of our outcomes including FFM and physical performance. Thus, our data indicate that the ACSM recommendation of 1.2-1.7g/kg is sufficient for athletes even during periods of ER. Of note, our study included a RT program specifically designed to maximize muscle hypertrophy. Hence, it is possible that without RT and the anabolic stimulus it provides, an athlete who undergoes a weight loss phase would need more protein than what is currently suggested (>2.2g/kg). Lastly, we found no significant detriments to

consuming more protein on any of our outcomes. Thus, consuming more protein than 1.2-1.7g/kg could be a plausible option for individuals who adhere to higher protein diets.

Strengths and Weaknesses

One of the main weaknesses of this project is its low statistical power due to the limited number of participants (n=12). Previous studies on the topic have had as much as 40 participants (Longland et al., 2016). Hence, to rectify this weakness, the study remains ongoing until the summer of 2023. An other weakness has to do with the nutritional intervention. A caloric restriction of 25% was chosen based on evidence suggesting that more aggressive deficits are more likely to cause decrements in physical performance (Garthe et al., 2011; Peos et al., 2018). However, because a margin of error is to be considered with any nutritional intervention that does not provide pre-made meals, it seems like an ER of 25% is too conservative for research purposes. In fact, our participants lost on average only ~1.3 kg. For this reason, it remains uncertain whether more protein would have an effect on our outcomes if they had lost weight significantly. Nonetheless, we did observe a significant reduction in REE indicating that our participants were for the most part in a state of ER. Lastly, the absence of evidence for training tonnage can be considered a weakness. It was initially a member of the research team that was responsible to track the weight and repetitions performed by participants on all the exercises during their training sessions. However, interruptions in regular laboratory activities because of the Covid-19 pandemic made it impossible to host all our participants for regular sessions causing us to lose track of the data. Moreover, undergraduate students who were assisting me on this project graduated and were no longer available to host the workouts. To resolve the issue, I provided an excel spreadsheet to all the participants with designated columns to input the loads and repetitions that were using during

their workouts. I asked the participants to keep track of the weight and repetitions performed. However, some participants never returned the spreadsheet after the intervention and others did not objectively track the loads and repetitions they performed during their workouts. Nonetheless, the changes in performance were similar across all groups suggesting that compliance to the training program was similar between groups.

Despite many limitations, this project also has many strengths. First, our protocol included three different groups with different intakes of protein. This allowed us to test a wide range of protein intake (~1.2g/kg up to ~2.2g/kg) without any values being overly distant from one another (i.e testing 1.2g/kg vs 2.2g/kg without a middle group). Second, we included both males and females in our study which distinguishes our sample size from that of previous studies (Pasiakos et al., 2013; Longland et al., 2016; Pearson et al., 2021). In addition, all of our participants were recreational or competitive athletes from various sports. We were able to design a protocol in which it was possible for our participants to maintain their regular practice schedule or sport related commitments throughout the intervention. This allowed the ecological value of the study to be maintained, which can be considered a strength. Finally, we used D_2O dilution to estimate TBW of our participants. Although the enriched saliva samples have yet to be analyzed because of Covid-19 related delays at the time of the deposition limit for this thesis, the D_2O dilution is a strength to our study. Deuterium oxide dilution is considered the gold standard to calculate TBW (Bila et al., 2016). This is relevant to our project because previous studies omit to report TBW or report values obtained from bioelectrical impedance analysis (Pasiakos et al., 2013; Longland et al., 2016; Pearson et al., 2021). It has previously been shown that bioelectrical impedance analysis tends to overestimate TBW which could lead to an overestimation of FFM (Bila et al., 2016). We obtained our FFM values from DXA analyses, hence, we will be able to deduct our TBW values

from the FFM and estimate the changes that occurred in tissue mass as the result of our intervention.

Implications for the Public and Future Research

Based on our data and that of others, it seems like athletes who consume between 1.2g/kg and 1.7g/kg of protein during periods of ER are likely to maximize the retention of FFM and physical performance so as long as RT is implemented (Pearson et al., 2021). Hence, normal weight individuals who undergo short-term periods of ER will most likely maximize their results with protein intakes in that range. However, it remains unclear whether protein needs to increase proportionally throughout an ER phase as an individual reaches very low levels of body fat (bodybuilders, physique athletes, individuals with anorexia etc.…). In fact, athletes in our study did not lose weight significantly. Hence, future research could replicate our protocol with a more aggressive restriction to see if the same conclusions are reached. Future research could also focus on middle-aged individuals (≥40 years old) who are susceptible to anabolic resistance to see if they would benefit from more protein during periods of ER. It would also be interesting to see if more total daily protein is needed for vegan or vegetarian athletes who undergo periods of ER.

In addition, the onset of AT was present in our study. It is unknown what role AT played on the outcomes of our study and whether it was responsible for the limited weight loss in our sample size. It is also unknown as to how early AT occurred in our study since we only measured REE at baseline and post intervention. Moreover, it is unclear as to whether AT subsides in athletes once energy balance is re-established. Hence, future studies that focus on identifying the onset of early AT in normal weight individuals and how to potentially limit it are needed.

Appendices

Weight Log Spreadsheet

Exercises/Days	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
A.						
Bulgarian Split squat						
Chest Press						
Leg Curl						
Machine Row						
Lateral Raise						
Russian Twist						
B.						
Incline chest Press						
Leg Press						
Upright Row						
Pronated Pull Down						
Barbell Curl						
Lying Triceps Extension						
Sit up						
C.						
Military Press						
1 leg RDL						
Supinated Pull Down						
Pec Deck						
Goblet Squat						
Incline Reverse Fly						

Day A. Training Sample

A. Full Body

1 - Safety Bar Bulgarian Split Squat

- Avec le "Safety Bar" sur vos épaules, faire des développés sur une jambe avec un pied sur un banc. Descendre en gardant le corps perpendiculaire au sol. Garder le dos droit et les abdos contractés.

Sem.	Séries	Rep	Charge (lb)	Repos
1	3	6 - 8		01:30
2	3	6 - 8		01:30
3	3	6 - 8		01:30
4	4	6 - 8		01:30
5	4	6 - 8		01:30
6	4	6 - 8		01:30

2 - Développé pectoraux sur machine

- Garder le dos à plat - Prise neutre ou marteau

Sem.	Séries	Rep	Charge (lb)	Repos
1	3	6 - 8		01:30
2	3	6 - 8		01:30
3	3	6 - 8		01:30
4	4	6 - 8		01:30
5	4	6 - 8		01:30
6	4	6 - 8		01:30

3 - Leg Curl sur machine

- Placer les chevilles sous les appuis. Faire des flexions de genoux en gardant un angle de 90° aux chevilles. Garder le dos droit et vos abdos tendus.

Sem.	Séries	Rep	Charge (lb)	Repos
1	3	10 - 12		01:00
2	3	10 - 12		01:00
3	3	10 - 12		01:00
4	3	10 - 12		01:00
5	3	10 - 12		01:00
6	3	10 - 12		01:00

4 - Ramer sur machine

- L'appui sur la poitrine, garder le dos droit, la tête haute et les épaules tirées vers l'arrière. Tirer jusqu'à ce que les coudes soient à 90°.

Sem.	Séries	Rep	Charge (lb)	Repos
1	3	10 - 12		01:00
2	3	10 - 12		01:00
3	3	10 - 12		01:00
4	3	10 - 12		01:00
5	3	10 - 12		01:00
6	3	10 - 12		01:00

5 - Élévation latéral des bras avec DB

- Les pieds à la largeur des épaules, garder les abdos tendus et la tête haute. Lever les coudes à la hauteur des épaules. Garder vos coudes légèrement fléchis. Prise neutre.

Sem.	Séries	Rep	Charge (lb)	Repos
1	3	20 - 25		01:00
2	3	20 - 25		01:00
3	3	20 - 25		01:00
4	3	20 - 25		01:00
5	3	20 - 25		01:00
6	3	20 - 25		01:00

6 - Plate Russian Twist

- En équilibre sur les fesses, les jambes allongées devant vous, déposer un poids (plate) d'un côté et de l'autre de vos hanches en tournant les épaules. Garder vos abdos contractés.

Sem.	Séries	Rep	Charge (lb)	Repos
1	3	20 - 25		01:00
2	3	20 - 25		01:00
3	3	20 - 25		01:00
4	3	20 - 25		01:00
5	3	20 - 25		01:00
6	3	20 - 25		01:00

Nutritional Template Sample

To help you ...
Keep track !

Equivalents recommended per day :

_____ Grain products : ☐☐☐☐☐☐☐☐☐☐☐☐☐
_____ Vegetables : ☐☐☐☐☐☐☐☐☐☐☐☐☐
_____ Fruits : ☐☐☐☐☐☐☐☐☐☐☐☐☐
_____ Dairy products : ☐☐☐☐☐☐☐☐☐☐☐☐☐
_____ Meat and substitutes : ☐☐☐☐☐☐☐☐☐☐☐☐☐
_____ Fat products : ☐☐☐☐☐☐☐☐☐☐☐☐☐

***Hint** : After each meal, fill in the number of squares that represent the number of equivalents that you consummed and compare your daily profil with your personal food plan.*

Ex : Breakfast : 175ml cereales ☑ Grain products : ☐☐☐☐☐☐☐☐☐☐☐☐☐
125 ml milk ☑ Dairy products : ☐☐☐☐☐☐☐☐☐☐☐☐☐
1 apple ☑ Fruits : ☐☐☐☐☐☐☐☐☐☐☐☐☐

Equivalents	Date :	Date :
Grain prod. :	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
Vegetables :	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
Fruits :	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
Dairy prod. :	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
Meat :	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
Fat prod. :	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Equivalents	Date :	Date :
Grain prod. :	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
Vegetables :	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
Fruits :	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
Dairy prod. :	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
Meat :	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐
Fat prod. :	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Vegetables 6 *equivalents/day*

One equivalent correspond to :

- Juice : tomatoes, vegetables, carrots,.....125 ml (1/2 cup)
- Winter squash, mixed vegetables, green peas, parsnips .....75 ml (1/3 cup)
- Artichoke heart, artichoke.....125 ml (1/2 moyen)
- Beats, carrots, Brussel sprouts, pumpkin puree, onions, leaks, peas, asparagus, brocolli, cabbage, cauliflower, summer squash, zucchini, endives, spinach (warm), chives, yellow or green beans, canned tomatoes, turnip.....125 ml (1/2 cup)
- Diced eggplant.....250 ml (1 cup)

Vegetables with high % of water

- Celery, mushrooms, cucumber, fiddle head, raw spinach, bean sprouts, alfalfa sprouts, radish, lettuce, peppers, tomatoes.....500 ml (2 cups)

Fruits 6 *equivalents/day*

One equivalent correspond to :

- Apricots, kiwi, dattes, fig, clementines, prunes.....2 fruits
- Fruit compotes, salads or jus and canned fruit (unsweetened).....125 ml (1/2 cup)
- Banana, mangoes, kaki, grapefruit, papaya.....1/2 fruit
- Nectarine, orange, peach, pear, apple, tangerine.....1 fruit
- Cantaloup.....1/4 fruit
- Honeydew.....1/8 fruit
- Strawberries, currant, rasberries, water melon, honeydew.....250 ml (1 cup)
- Rhubarbe.....500 ml (2 cups)

Grain products 5 *equivalents/day*

One equivalent correspond to :

- Toasted bread, dry crackers (all kinds).....2 units
- Soda Crackers, Melba.....5 units
- Breakfast cereals (warm or ready to serve, little or no sugar).....30 g (3/4 cup)
- Rice, couscous, pasta, mashed potatoes, corn.....125 ml (1/2 cup)
- Popcorn (no butter).....750 ml (3 cups)
- Hamburger or hot dogs buns, bagel English muffin, Keiser, pita.....1/2 unit
- Sliced bread, small salad bread.....1 unit
- Baguette 1½ slice.....4 units
- Pasta soup.....250 ml (1 cup)
- Pea soup.....125 ml (1/2 cup)
- _____
- _____
- _____

Dairy Products 3 *equivalents/day*

One equivalent correspond to

- Milk 2%, 1% or skimmed.....250 ml
- Yogurt < 2% m.f.....175 ml
- Unswettened evaporated milk (Carnation).....100 ml
- Ice cream.....250 ml
- Powdered milk.....60 ml
- _____
- _____
- _____
- _____

Meat & substitutes 12 *equivalents/day*

One equivalent correspond to :

- Cheese (skimmed) (7 à 18% m.f.).....30 g (1 ounce)
- Regular cheese (> 20% m.f.)*.....30 g (1 ounce)
- Cottage cheese 1%, 2% m.f. or ricotta (skimmed).....60 ml (1/4 cup)
- Meat, poultry, fish.....30 g (1 ounce)
- Shrimp.....5 big, 18 small
- Egg.....1 unit
- Peanut butter.....15 ml (1 table sp.)
- Legumes°.....125 ml (1/2 cup)
- Tofu.....125 ml (1/2 cup)
- Whey- 1 scoop = 3 portions
- _____

* Substract 2 fats from your fat equivalents

° Substract 1 bread from your bread equivalents

Fat products 6 *equivalents/day*

One equivalent correspond to (5 g of fat products) :

- Butter, margarine, mayonnaise, oil.....5 ml (1 tea sp.)
- Cream cheese, light margarine, light mayonnaise, light salad dressing, vinaigrette.....15 ml (1 table sp.)
- Avocado.....1/8 unit
- Olives.....8 olives
- Nuts and grains.....15 ml (1 table sp.)
- _____

Limited consumption!!

- Seasonning
- Bouillons and Consommés
- Diet soft drinks
- Tea, coffee, herbal tea
- Soya sauce, Worcestershire
- Dry or prepared mustard, Ketchup, Vinaigar
- Mayonnaise and dressing ULTRA LOW IN FAT

References

American College of Sports Medicine ed., 2013. ACSM's health-related physical fitness assessment manual. Lippincott Williams & Wilkins.

Ahtiainen, J. P., Pakarinen, A., Alen, M., Kraemer, W. J., & H�kkinen, K. (2003). Muscle hypertrophy, hormonal adaptations and strength development during strength training in strength-trained and untrained men. European Journal of Applied Physiology, 89(6), 555–563. https://doi.org/10.1007/s00421-003-0833-3

Bila, W. C., de Freitas, A. E., Galdino, A. S., Ferriolli, E., Pfrimer, K., & Lamounier, J. A. (2016). Deuterium oxide dilution and body composition in overweight and obese schoolchildren aged 6-9 years. *Jornal de Pediatria (Versão em Português)*, *92*(1), 46-52.

Blond, E., Maitrepierre, C., Normand, S., Sothier, M., Roth, H., Goudable, J., & Laville, M. (2011). A new indirect calorimeter is accurate and reliable for measuring basal energy expenditure, thermic effect of food and substrate oxidation in obese and healthy subjects. *e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism*, *6*(1), e7-e15.

Burkholder, T., J. (2007). Mechanotransduction in skeletal muscle. Frontiers in Bioscience, 12(1), 174. https://doi.org/10.2741/2057

Butterfield, G. E., & Calloway, D. H. (1984). Physical activity improves protein utilization in young men. British Journal of Nutrition, 51(02), 171. https://doi.org/10.1079/BJN19840021

Calcagno, M., Kahleova, H., Alwarith, J., Burgess, N. N., Flores, R. A., Busta, M. L., & Barnard, N. D. (2019). The Thermic Effect of Food: A Review. Journal of the American College of Nutrition, 38(6), 547–551. https://doi.org/10.1080/07315724.2018.1552544

Campbell, B. I., Aguilar, D., Conlin, L., Vargas, A., Schoenfeld, B. J., Corson, A., Gai, C., Best, S., Galvan, E., & Couvillion, K. (2018). Effects of High Versus Low Protein Intake on Body Composition and Maximal Strength in Aspiring Female Physique Athletes Engaging in an 8-Week Resistance Training Program. International Journal of Sport Nutrition and Exercise Metabolism, 28(6), 580–585. https://doi.org/10.1123/ijsnem.2017-0389

Chittenden, R. H. (1904). *Physiological economy in nutrition*. Stokes

Clarkson, P. M., & Hubal, M. J. (2002). Exercise-Induced Muscle Damage in Humans: American Journal of Physical Medicine & Rehabilitation, 81(Supplement), S52–S69. https://doi.org/10.1097/00002060-200211001-00007

Coffey, V. G., Zhong, Z., Shield, A., Canny, B. J., Chibalin, A. V., Zierath, J. R., & Hawley, J. A. (2006). Early signaling responses to divergent exercise stimuli in skeletal muscle from well-trained humans. The FASEB Journal, 20(1), 190–192. https://doi.org/10.1096/fj.05-4809fje

Cooper, J. A., Watras, A. C., O'Brien, M. J., Luke, A., Dobratz, J. R., Earthman, C. P., & Schoeller, D. A. (2009). Assessing validity and reliability of resting metabolic rate in six gas analysis systems. *Journal of the American Dietetic Association*, *109*(1), 128-132.

Doucet, E., St-Pierre, S., Alméras, N., Després, J.-P., Bouchard, C., & Tremblay, A. (2001). Evidence for the existence of adaptive thermogenesis during weight loss. British Journal of Nutrition, 85(6), 715–723. https://doi.org/10.1079/BJN2001348

DRI. (2005). The Institute of Medicine, Food and Nutrition Board, dietary reference intakes: energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids

Dulloo, A. G., & Jacquet, J. (1998). Adaptive reduction in basal metabolic rate in response to food deprivation in humans: A role for feedback signals from fat stores. The American Journal of Clinical Nutrition, 68(3), 599–606. https://doi.org/10.1093/ajcn/68.3.599

Elia, M. (1992). Effect of starvation and very low calorie diets on protein-energy interrelationships in lean and obese subjects. *Protein-energy interactions*, *249*.

Elia, M., Stubbs, R. J., & Henry, C. J. K. (1999). Differences in Fat, Carbohydrate, and Protein Metabolism between Lean and Obese Subjects Undergoing Total Starvation. Obesity Research, 7(6), 597–604. https://doi.org/10.1002/j.1550-8528.1999.tb00720.x

Faigenbaum, A. D. (2009). OVERTRAINING IN YOUNG ATHLETES: How Much Is Too Much? ACSM'S Health & Fitness Journal, 13(4), 8–13. https://doi.org/10.1249/FIT.0b013e3181aae0a0

Figueiredo, V. C., de Salles, B. F., & Trajano, G. S. (2018). Volume for Muscle Hypertrophy and Health Outcomes: The Most Effective Variable in Resistance Training. Sports Medicine, 48(3), 499–505. https://doi.org/10.1007/s40279-017-0793-0

Fry, C. S., Glynn, E. L., Drummond, M. J., Timmerman, K. L., Fujita, S., Abe, T., Dhanani, S., Volpi, E., & Rasmussen, B. B. (2010). Blood flow restriction exercise stimulates mTORC1 signaling and muscle protein synthesis in older men. Journal of Applied Physiology, 108(5), 1199–1209. https://doi.org/10.1152/japplphysiol.01266.2009

Fujita, S., Abe, T., Drummond, M. J., Cadenas, J. G., Dreyer, H. C., Sato, Y., Volpi, E., & Rasmussen, B. B. (2007). Blood flow restriction during low-intensity resistance exercise increases S6K1 phosphorylation and muscle protein synthesis. Journal of Applied Physiology, 103(3), 903–910. https://doi.org/10.1152/japplphysiol.00195.2007

Garthe, I., Raastad, T., Refsnes, P. E., Koivisto, A., & Sundgot-Borgen, J. (2011). Effect of Two Different Weight-Loss Rates on Body Composition and Strength and Power-Related Performance in Elite Athletes. International Journal of Sport Nutrition and Exercise Metabolism, 21(2), 97–104. https://doi.org/10.1123/ijsnem.21.2.97

Goodman, C. A., Frey, J. W., Mabrey, D. M., Jacobs, B. L., Lincoln, H. C., You, J.-S., & Hornberger, T. A. (2011). The role of skeletal muscle mTOR in the regulation of mechanical load-induced growth: The role of mTOR in the regulation of mechanical load-induced growth. The Journal of Physiology, 589(22), 5485–5501. https://doi.org/10.1113/jphysiol.2011.218255

Grgic, J., Mcllvenna, L. C., Fyfe, J. J., Sabol, F., Bishop, D. J., Schoenfeld, B. J., & Pedisic, Z. (2019). Does Aerobic Training Promote the Same Skeletal Muscle Hypertrophy as Resistance Training? A Systematic Review and Meta-Analysis. Sports Medicine, 49(2), 233–254. https://doi.org/10.1007/s40279-018-1008-z

Halton, T. L., & Hu, F. B. (2004). The Effects of High Protein Diets on Thermogenesis, Satiety and Weight Loss: A Critical Review. Journal of the American College of Nutrition, 23(5), 373–385. https://doi.org/10.1080/07315724.2004.10719381

Harber, M. P., Crane, J. D., Dickinson, J. M., Jemiolo, B., Raue, U., Trappe, T. A., & Trappe, S. W. (2009). Protein synthesis and the expression of growth-related genes are altered by running in human vastus lateralis and soleus muscles. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 296(3), R708–R714. https://doi.org/10.1152/ajpregu.90906.2008

Haun, C. T., Vann, C. G., Roberts, B. M., Vigotsky, A. D., Schoenfeld, B. J., & Roberts, M. D. (2019). A Critical Evaluation of the Biological Construct Skeletal Muscle Hypertrophy:

Size Matters but So Does the Measurement. Frontiers in Physiology, 10, 247. https://doi.org/10.3389/fphys.2019.00247

Heinitz, S., Hollstein, T., Ando, T., Walter, M., Basolo, A., Krakoff, J., Votruba, S. B., & Piaggi, P. (2020). Early adaptive thermogenesis is a determinant of weight loss after six weeks of caloric restriction in overweight subjects. Metabolism, 110, 154303. https://doi.org/10.1016/j.metabol.2020.154303

Helms, E. R., Zinn, C., Rowlands, D. S., Naidoo, R., & Cronin, J. (2015). High-Protein, Low-Fat, Short-Term Diet Results in Less Stress and Fatigue Than Moderate-Protein, Moderate-Fat Diet During Weight Loss in Male Weightlifters: A Pilot Study. International Journal of Sport Nutrition and Exercise Metabolism, 25(2), 163–170. https://doi.org/10.1123/ijsnem.2014-0056

Hintze, L. J., Goldfield, G., Seguin, R., Damphousse, A., Riopel, A., & Doucet, É. (2019). The rate of weight loss does not affect resting energy expenditure and appetite sensations differently in women living with overweight and obesity. Physiology & Behavior, 199, 314–321. https://doi.org/10.1016/j.physbeh.2018.11.032

Hintze, L. J., Messier, V., Lavoie, M.-È., Brochu, M., Lavoie, J.-M., Prud'homme, D., Rabasa-Lhoret, R., & Doucet, É. (2018). A one-year resistance training program following weight loss has no significant impact on body composition and energy expenditure in postmenopausal women living with overweight and obesity. Physiology & Behavior, 189, 99–106. https://doi.org/10.1016/j.physbeh.2018.03.014

Huovinen, H. T., Hulmi, J. J., Isolehto, J., Kyröläinen, H., Puurtinen, R., Karila, T., Mackala, K., & Mero, A. A. (2015a). Body Composition and Power Performance Improved After Weight Reduction in Male Athletes Without Hampering Hormonal Balance: Journal of

Strength and Conditioning Research, 29(1), 29–36. https://doi.org/10.1519/JSC.0000000000000619

Huovinen, H. T., Hulmi, J. J., Isolehto, J., Kyröläinen, H., Puurtinen, R., Karila, T., Mackala, K., & Mero, A. A. (2015b). Body Composition and Power Performance Improved After Weight Reduction in Male Athletes Without Hampering Hormonal Balance. Journal of Strength and Conditioning Research, 29(1), 29–36. https://doi.org/10.1519/JSC.0000000000000619

Israetel, M., Feather, J., Faleiro, T. V., & Juneau, C.-E. (2020). Mesocycle Progression in Hypertrophy: Volume Versus Intensity. Strength & Conditioning Journal, 42(5), 2–6. https://doi.org/10.1519/SSC.0000000000000518

INTRODUCTION TO BODY COMPOSITION ASSESSMENT USING THE DEUTERIUM DILUTION TECHNIQUE WITH ANALYSIS OF SALIVA SAMPLES BY FOURIER TRANSFORM INFRARED SPECTROMETRY, n.d.

JMVelthuis-te Wierik, E., Hoogzaad, L. V., Van den Berg, H., & Schaafsma, G. (1994). Effects of moderate energy restriction on physical performance and substrate utilization in non-obese men. *International journal of sports medicine*, *15*(08), 478-484.

Kato, H., Suzuki, K., Bannai, M., & Moore, D. R. (2016). Protein Requirements Are Elevated in Endurance Athletes after Exercise as Determined by the Indicator Amino Acid Oxidation Method. PLOS ONE, 11(6), e0157406. https://doi.org/10.1371/journal.pone.0157406

Konopka, A. R., & Harber, M. P. (2014). Skeletal Muscle Hypertrophy After Aerobic Exercise Training. Exercise and Sport Sciences Reviews, 42(2), 53–61. https://doi.org/10.1249/JES.0000000000000007

Koopman, R., Zorenc, A. H. G., Gransier, R. J. J., Cameron-Smith, D., & van Loon, L. J. C. (2006). Increase in S6K1 phosphorylation in human skeletal muscle following resistance exercise occurs mainly in type II muscle fibers. American Journal of Physiology-Endocrinology and Metabolism, 290(6), E1245–E1252. https://doi.org/10.1152/ajpendo.00530.2005

Kraemer, W. J., Adams, K., Cafarelli, E., Dudley, G. A., Dooly, C., Feigenbaum, M. S., ... & Triplett-McBride, T. (2002). American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Medicine and science in sports and exercise*, *34*(2), 364-380.

Krzysztofik, Wilk, Wojdała, & Gołaś. (2019). Maximizing Muscle Hypertrophy: A Systematic Review of Advanced Resistance Training Techniques and Methods. International Journal of Environmental Research and Public Health, 16(24), 4897. https://doi.org/10.3390/ijerph16244897

Lasevicius, T., Ugrinowitsch, C., Schoenfeld, B. J., Roschel, H., Tavares, L. D., De Souza, E. O., Laurentino, G., & Tricoli, V. (2018). Effects of different intensities of resistance training with equated volume load on muscle strength and hypertrophy. European Journal of Sport Science, 18(6), 772–780. https://doi.org/10.1080/17461391.2018.1450898

Lee, J. H., & Jun, H.-S. (2019). Role of Myokines in Regulating Skeletal Muscle Mass and Function. Frontiers in Physiology, 10, 42. https://doi.org/10.3389/fphys.2019.00042

Lemon, P. W. R. (2000). Beyond the Zone: Protein Needs of Active Individuals. Journal of the American College of Nutrition, 19(sup5), 513S-521S. https://doi.org/10.1080/07315724.2000.10718974

Lim, C., Nunes, E. A., Currier, B. S., Mcleod, J. C., Thomas, A. C. Q., & Phillips, S. M. (2022). An Evidence-Based Narrative Review of Mechanisms of Resistance Exercise–Induced Human Skeletal Muscle Hypertrophy. Medicine & Science in Sports & Exercise, 54(9), 1546–1559. https://doi.org/10.1249/MSS.0000000000002929

Liu, G. Y., & Sabatini, D. M. (2020). MTOR at the nexus of nutrition, growth, ageing and disease. Nature Reviews Molecular Cell Biology, 21(4), 183–203. https://doi.org/10.1038/s41580-019-0199-y

Loenneke, J. P., Wilson, G. J., & Wilson, J. M. (2010). A Mechanistic Approach to Blood Flow Occlusion. International Journal of Sports Medicine, 31(01), 1–4. https://doi.org/10.1055/s-0029-1239499

Longland, T. M., Oikawa, S. Y., Mitchell, C. J., Devries, M. C., & Phillips, S. M. (2016). Higher compared with lower dietary protein during an energy deficit combined with intense exercise promotes greater lean mass gain and fat mass loss: A randomized trial. The American Journal of Clinical Nutrition, 103(3), 738–746. https://doi.org/10.3945/ajcn.115.119339

Lowe, D. A., & Alway, S. E. (n.d.). Stretch-induced myogenin, MyoD, and MRF4 expression and acute hypertrophy in quail slow-tonic muscle are not dependent upon satellite cell proliferation. 9.

Marshall, P. W. M., McEwen, M., & Robbins, D. W. (2011). Strength and neuromuscular adaptation following one, four, and eight sets of high intensity resistance exercise in trained males. European Journal of Applied Physiology, 111(12), 3007–3016. https://doi.org/10.1007/s00421-011-1944-x

McCoy, R. H., Meyer, C. E., & Rose, W. C. (1935). FEEDING EXPERIMENTS WITH MIXTURES OF HIGHLY PURIFIED AMINO ACIDS. Journal of Biological Chemistry, 112(1), 283–302. https://doi.org/10.1016/S0021-9258(18)74986-7

McGinley, C., Shafat, A., & Donnelly, A. E. (2009). Does Antioxidant Vitamin Supplementation Protect against Muscle Damage?: Sports Medicine, 39(12), 1011–1032. https://doi.org/10.2165/11317890-000000000-00000

McIver, C. M., Wycherley, T. P., & Clifton, P. M. (2012). MTOR signaling and ubiquitin-proteosome gene expression in the preservation of fat free mass following high protein, calorie restricted weight loss. Nutrition & Metabolism, 9(1), 83. https://doi.org/10.1186/1743-7075-9-83

Melin, A. K., Heikura, I. A., Tenforde, A., & Mountjoy, M. (2019a). Energy Availability in Athletics: Health, Performance, and Physique. International Journal of Sport Nutrition and Exercise Metabolism, 29(2), 152–164. https://doi.org/10.1123/ijsnem.2018-0201

Melin, A. K., Heikura, I. A., Tenforde, A., & Mountjoy, M. (2019b). Energy Availability in Athletics: Health, Performance, and Physique. International Journal of Sport Nutrition and Exercise Metabolism, 29(2), 152–164. https://doi.org/10.1123/ijsnem.2018-0201

Mettler, S., Mitchell, N., & Tipton, K. D. (2010). Increased Protein Intake Reduces Lean Body Mass Loss during Weight Loss in Athletes. Medicine & Science in Sports & Exercise, 42(2), 326–337. https://doi.org/10.1249/MSS.0b013e3181b2ef8e

Mitchell, L., Slater, G., Hackett, D., Johnson, N., & O'connor, H. (2018). Physiological implications of preparing for a natural male bodybuilding competition. European Journal of Sport Science, 18(5), 619–629. https://doi.org/10.1080/17461391.2018.1444095

Moon, J. R., Tobkin, S. E., Roberts, M. D., Dalbo, V. J., Kerksick, C. M., Bemben, M. G., Cramer, J. T., & Stout, J. R. (2008). Total body water estimations in healthy men and women using bioimpedance spectroscopy: A deuterium oxide comparison. Nutrition & Metabolism, 5(1), 7. https://doi.org/10.1186/1743-7075-5-7

Moore, J. L., Travis, S. K., Lee, M. L., & Stone, M. H. (2019). Making Weight: Maintaining Body Mass for Weight Class Barbell Athletes. Strength & Conditioning Journal, 41(6), 110–114. https://doi.org/10.1519/SSC.0000000000000503

Morton, R. W., Murphy, K. T., McKellar, S. R., Schoenfeld, B. J., Henselmans, M., Helms, E., Aragon, A. A., Devries, M. C., Banfield, L., Krieger, J. W., & Phillips, S. M. (2018). A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. British Journal of Sports Medicine, 52(6), 376–384. https://doi.org/10.1136/bjsports-2017-097608

Murphy, C. H., Hector, A. J., & Phillips, S. M. (2015). Considerations for protein intake in managing weight loss in athletes. European Journal of Sport Science, 15(1), 21–28. https://doi.org/10.1080/17461391.2014.936325

Nelson, K. M., Weinsier, R. L., Long, C. L., & Schutz, Y. (1992). Prediction of resting energy expenditure from fat-free mass and fat mass. The American Journal of Clinical Nutrition, 56(5), 848–856. https://doi.org/10.1093/ajcn/56.5.848

Pardue, A., Trexler, E. T., & Sprod, L. K. (2017). Case Study: Unfavorable But Transient Physiological Changes During Contest Preparation in a Drug-Free Male Bodybuilder. International Journal of Sport Nutrition and Exercise Metabolism, 27(6), 550–559. https://doi.org/10.1123/ijsnem.2017-0064

Pasiakos, S. M., Cao, J. J., Margolis, L. M., Sauter, E. R., Whigham, L. D., McClung, J. P., ... & Young, A. J. (2013). Effects of high-protein diets on fat-free mass and muscle protein synthesis following weight loss: a randomized controlled trial. *The FASEB Journal*, *27*(9), 3837-3847.

Pearson, A. G., Alexander, L., Witard, O. C., Coughlin, T. E., Tipton, K. D., & Walshe, I. H. (2021). A hypoenergetic diet with decreased protein intake does not reduce lean body mass in trained females. European Journal of Applied Physiology, 121(3), 771–781. https://doi.org/10.1007/s00421-020-04555-7

Peronnet, F., & Massicotte, D. (1991). Table of nonprotein respiratory quotient: an update. *Can J Sport Sci*, *16*(1), 23-29.

Péronnet, F., & Haman, F. (2019). Low capacity to oxidize fat and body weight. Obesity Reviews, 20(10), 1367–1383. https://doi.org/10.1111/obr.12910

Petrizzo, J., DiMenna, F. J., Martins, K., Wygand, J., & Otto, R. M. (2017). Case Study: The Effect of 32 Weeks of Figure-Contest Preparation on a Self-Proclaimed Drug-Free Female's Lean Body and Bone Mass. International Journal of Sport Nutrition and Exercise Metabolism, 27(6), 543–549. https://doi.org/10.1123/ijsnem.2016-0313

Phillips, S. M., Glover, E. I., & Rennie, M. J. (2009). Alterations of protein turnover underlying disuse atrophy in human skeletal muscle. Journal of Applied Physiology, 107(3), 645–654. https://doi.org/10.1152/japplphysiol.00452.2009

Phillips, S. M., & Van Loon, L. J. C. (2011). Dietary protein for athletes: From requirements to optimum adaptation. Journal of Sports Sciences, 29(sup1), S29–S38. https://doi.org/10.1080/02640414.2011.619204

Pileggi, C. A., Hooks, B. G., McPherson, R., Dent, R. R. M., & Harper, M.-E. (2022). Targeting skeletal muscle mitochondrial health in obesity. Clinical Science, 136(14), 1081–1110. https://doi.org/10.1042/CS20210506

Price, G. M., Halliday, D., Pacy, P. J., Quevedo, M. R., & Millward, D. J. (1994). Nitrogen Homoeostasis in man: Influence of Protein Intake on the Amplitude of Diurnal Cycling of Body Nitrogen. Clinical Science, 86(1), 91–102. https://doi.org/10.1042/cs0860091

Rand, W. M., Pellett, P. L., & Young, V. R. (2003). Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. The American Journal of Clinical Nutrition, 77(1), 109–127. https://doi.org/10.1093/ajcn/77.1.109

Rankin, J. W. (2002). Weight Loss and Gain in Athletes: Current Sports Medicine Reports, 1(4), 208–213. https://doi.org/10.1249/00149619-200208000-00004

Rennie, M. J. (1999). Physical exertion, amino acid and protein metabolism and protein requirements. *The role of protein and amino acids in sustaining and enhancing performance*, 243-253.

Rennie, M. J., & Tipton, K. D. (2000). P ROTEIN AND A MINO A CID M ETABOLISM D URING AND A FTER E XERCISE AND THE E FFECTS OF N UTRITION. Annual Review of Nutrition, 20(1), 457–483. https://doi.org/10.1146/annurev.nutr.20.1.457

Rodenburg, J. B., de Boer, R. W., Schiereck, P., van Echteld, C. J. A., & Bär, P. R. (1994). Changes in phosphorus compounds and water content in skeletal muscle due to eccentric exercise. European Journal of Applied Physiology and Occupational Physiology, 68(3), 205–213. https://doi.org/10.1007/BF00376768

Rose, W. C. (1968). 11. THE SEQUENCE OF EVENTS LEADING TO THE ESTABLISHMENT OF THE AMINO ACID NEEDS OF MAN. AMINO ACIDS, 8.

Sabouri, M. (2022). Effect of eccentric and concentric contraction mode on myogenic regulatory factors expression in human vastus lateralis muscle. Journal of Muscle Research and Cell Motility, 12.

Sardeli, A., Komatsu, T., Mori, M., Gáspari, A., & Chacon-Mikahil, M. (2018). Resistance Training Prevents Muscle Loss Induced by Caloric Restriction in Obese Elderly Individuals: A Systematic Review and Meta-Analysis. Nutrients, 10(4), 423. https://doi.org/10.3390/nu10040423

Schoenfeld, B. (2000). Repetitions and Muscle Hypertrophy. Strength and Conditioning Journal, 3.

Schoenfeld, B., Grgic, J., Haun, C., Itagaki, T., & Helms, E. (2019). Calculating Set-Volume for the Limb Muscles with the Performance of Multi-Joint Exercises: Implications for Resistance Training Prescription. Sports, 7(7), 177. https://doi.org/10.3390/sports7070177

Schoenfeld, B. J. (2010a). The Mechanisms of Muscle Hypertrophy and Their Application to Resistance Training. Journal of Strength and Conditioning Research, 24(10), 2857–2872. https://doi.org/10.1519/JSC.0b013e3181e840f3

Schoenfeld, B. J. (2010b). The Mechanisms of Muscle Hypertrophy and Their Application to Resistance Training. Journal of Strength and Conditioning Research, 24(10), 2857–2872. https://doi.org/10.1519/JSC.0b013e3181e840f3

Schoenfeld, B. J. (2012). Does Exercise-Induced Muscle Damage Play a Role in Skeletal Muscle Hypertrophy? Journal of Strength and Conditioning Research, 26(5), 1441–1453. https://doi.org/10.1519/JSC.0b013e31824f207e

Schoenfeld, B. J. (2013). Potential Mechanisms for a Role of Metabolic Stress in Hypertrophic Adaptations to Resistance Training. Sports Medicine, 43(3), 179–194. https://doi.org/10.1007/s40279-013-0017-1

Schoenfeld, B. J., Alto, A., Grgic, J., Tinsley, G., Haun, C. T., Campbell, B. I., Escalante, G., Sonmez, G. T., Cote, G., Francis, A., & Trexler, E. T. (2020). Alterations in Body Composition, Resting Metabolic Rate, Muscular Strength, and Eating Behavior in Response to Natural Bodybuilding Competition Preparation: A Case Study. Journal of Strength and Conditioning Research, 34(11), 3124–3138. https://doi.org/10.1519/JSC.0000000000003816

Schoenfeld, B. J., Contreras, B., Krieger, J., Grgic, J., Delcastillo, K., Belliard, R., & Alto, A. (2019). Resistance Training Volume Enhances Muscle Hypertrophy but Not Strength in Trained Men. Medicine & Science in Sports & Exercise, 51(1), 94–103. https://doi.org/10.1249/MSS.0000000000001764

Schoenfeld, B. J., Grgic, J., Ogborn, D., & Krieger, J. W. (2017). Strength and Hypertrophy Adaptations Between Low- vs. High-Load Resistance Training: A Systematic Review and Meta-analysis. Journal of Strength and Conditioning Research, 31(12), 3508–3523. https://doi.org/10.1519/JSC.0000000000002200

Schoenfeld, B. J., Ogborn, D., & Krieger, J. W. (2017). Dose-response relationship between weekly resistance training volume and increases in muscle mass: A systematic review and meta-analysis. Journal of Sports Sciences, 35(11), 1073–1082. https://doi.org/10.1080/02640414.2016.1210197

Schoenfeld, B. J., Peterson, M. D., Ogborn, D., Contreras, B., & Sonmez, G. T. (2015). Effects of Low- vs. High-Load Resistance Training on Muscle Strength and Hypertrophy in

Well-Trained Men. Journal of Strength and Conditioning Research, 29(10), 2954–2963. https://doi.org/10.1519/JSC.0000000000000958

Scott, B. R., Duthie, G. M., Thornton, H. R., & Dascombe, B. J. (2016). Training Monitoring for Resistance Exercise: Theory and Applications. Sports Medicine, 46(5), 687–698. https://doi.org/10.1007/s40279-015-0454-0

Sertel, O., Dogdas, B., Chiu, C. S., & Gurcan, M. N. (2011). Microscopic image analysis for quantitative characterization of muscle fiber type composition. Computerized Medical Imaging and Graphics, 35(7–8), 616–628. https://doi.org/10.1016/j.compmedimag.2011.01.009

Shamim, B., Devlin, B. L., Timmins, R. G., Tofari, P., Lee Dow, C., Coffey, V. G., Hawley, J. A., & Camera, D. M. (2018). Adaptations to Concurrent Training in Combination with High Protein Availability: A Comparative Trial in Healthy, Recreationally Active Men. Sports Medicine, 48(12), 2869–2883. https://doi.org/10.1007/s40279-018-0999-9

Sundgot-Borgen, J., & Garthe, I. (2011). Elite athletes in aesthetic and Olympic weight-class sports and the challenge of body weight and body compositions. Journal of Sports Sciences, 29(sup1), S101–S114. https://doi.org/10.1080/02640414.2011.565783

Tinsley, G. M., Trexler, E. T., Smith-Ryan, A. E., Paoli, A., Graybeal, A. J., Campbell, B. I., & Schoenfeld, B. J. (2019). Changes in Body Composition and Neuromuscular Performance Through Preparation, 2 Competitions, and a Recovery Period in an Experienced Female Physique Athlete. Journal of Strength and Conditioning Research, 33(7), 1823–1839. https://doi.org/10.1519/JSC.0000000000002758

Tipton, K. D. (2011). Efficacy and consequences of very-high-protein diets for athletes and exercisers. Proceedings of the Nutrition Society, 70(2), 205–214. https://doi.org/10.1017/S0029665111000024

Trexler, E. T., Smith-Ryan, A. E., & Norton, L. E. (2014). Metabolic adaptation to weight loss: Implications for the athlete. 7.

VanHeest, J. L., Rodgers, C. D., Mahoney, C. E., & De Souza, M. J. (2014). Ovarian suppression impairs sport performance in junior elite female swimmers. *Medicine and science in sports and exercise*, *46*(1), 156-166.

Vickery, H. Bradford., & Schmidt, C. L. A. (1931). The History of the Discovery of the Amino Acids. Chemical Reviews, 9(2), 169–318. https://doi.org/10.1021/cr60033a001

Vigh-Larsen, J. F., Ørtenblad, N., Spriet, L. L., Overgaard, K., & Mohr, M. (2021). Muscle Glycogen Metabolism and High-Intensity Exercise Performance: A Narrative Review. Sports Medicine, 51(9), 1855–1874. https://doi.org/10.1007/s40279-021-01475-0

von Liebig, J. (1841). *Organic chemistry in its application to agriculture and physiology*.

Wilkinson, S. B., Phillips, S. M., Atherton, P. J., Patel, R., Yarasheski, K. E., Tarnopolsky, M. A., & Rennie, M. J. (2008). Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle: Protein synthesis, resistance and endurance exercise. The Journal of Physiology, 586(15), 3701–3717. https://doi.org/10.1113/jphysiol.2008.153916

Wu, G. (2016). Dietary protein intake and human health. Food & Function, 7(3), 1251–1265. https://doi.org/10.1039/C5FO01530H