INTEGRATION OF TOTAL DAILY PROTEIN INTAKE AND TIMING OF PROTEIN SUPPLEMENTATION ON MUSCLE ANABOLISM DURING SIMULATED ELITE ATHLETE TRAINING IN FIT YOUNG MALES

A Dissertation

by

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ABSTRACT

A great deal of variation exists in recommendations of total daily protein intake and timing of supplementation for athletes. The most widely accepted recommendations promote protein intakes for strength and power athletes of 1.6-1.8 grams/kg/day and post-exercise protein supplementation. To our knowledge, no studies have investigated an interaction between supplementation timing and total daily needs in athletic populations. In an effort to determine optimal protein intake and supplementation strategies for athletic populations in a stable training phase, a double blind randomized controlled trial was conducted on 46 young, trained males (21.8±3.1 yr, 182.2±6.2 cm, 83.5±13.6 kg). Subjects underwent a two-week familiarization period followed by the two-week intervention period, both consisting of concurrent sprint interval and resistance exercise with nutritional interventions of low (LO = 1.3 g/kg total mass/day, 1.9 g/kg lean mass/day) and high (HI = 2.2 g/kg total mass/day, 2.7 g/kg lean mass/day) daily protein intake and whey protein supplementation either immediately (IPE) or three hours delayed (DPE) post-exercise. An age and activity matched control group (CON) completed food and activity logs but continued their normal diet (1.6 g/kg total mass/day, 2.1 g/kg lean mass/day) and exercise regimens and did not perform exercise on the experimental day. Tests of body composition, power, and strength were conducted before and after the intervention period. Cumulative muscle protein synthesis (C-MPS) was determined using deuterium stable isotope labeling (70%$^{2}H_2O$, 3ml/kg) to measure myofibrillar fractional synthetic rates (myoFSR) during the 24-hour post-exercise window.

A two-way ANOVA (total protein x timing) showed no difference in myoFSR among groups. No differences in total body %fat or lean mass were found, but changes in thigh %fat ($p=0.002$), total thigh fat mass ($p<0.001$), and thigh cross section fat mass ($p=0.049$) were significantly greater in LO/DPE compared to CON. Knee extension one-repetition-maximum was significantly greater at follow-up in LO/DPE compared to CON ($p=0.006$) and change in knee extension 1RM was greater in HI/DPE compared to CON ($p=0.006$). Thus, trained individuals undergoing simulated elite athlete training exhibited no significant differences in muscle protein synthesis, lean mass accretion, or performance measures regardless of total daily protein intake or supplementation timing strategy.

DEDICATION

I dedicate this dissertation to my dogs Tex and Hank, who kept me (relatively) sane on my academic journey, and to my family and friends who supported me completely.

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NOMENCLATURE

RDA	Recommended Daily Allowance
MPS	Muscle Protein Synthesis
MPB	Muscle Protein Breakdown
RE	Resistance Exercise (acute)
RET	Resistant Exercise Training (chronic)
C-MPS	Cumulative Muscle Protein Synthesis
AUC	Area Under Curve
FSR	Fractional Synthetic Rate
myoFSR	Myofibrillar Fractional Synthetic Rate
LBM	Lean Body Mass
D2O	Deuterium Oxide
1RM	1 Repetition Maximum
3RM	3 Repetition Maximum
LO	Low total daily protein
HI	High total daily protein
DPE	Delayed Post-Exercise supplementation
IPE	Immediate Post-Exercise supplementation
LO/DPE	Low Protein/Delayed Post-Exercise
LO/IPE	Low Protein/Immediate Post-Exercise
HI/DPE	High Protein/Delayed Post-Exercise
HI/IPE	High Protein/Immediate Post-Exercise
CON	Control
SSS	Stanford Sleepiness Scale
PSQI	Pittsburgh Sleep Quality Index
MAQ	Modified Activity Questionnaire
BD	Blood Draw
MB	Muscle Biopsy
RPE	Rating of Perceived Exertion
DEXA	Dual-X-ray Absorptiometry
FAM	Familiarization
INT	Intervention

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CHAPTER I

INTRODUCTION

While a standard for total daily protein intake for athletes and active individuals is generally accepted, recommendations for athletes have in fact fluctuated between slightly above to intakes 3 times sedentary levels. Two contrary hypotheses exist in the literature, the first being that active individuals and athletes have higher protein requirements than sedentary populations (61, 104), and the second proposing that adaptation occurs over time in such individuals, so that they do not have a significantly increased daily protein need (18, 19, 75, 85, 86, 102, 110). The U.S. and Canadian dietary reference intakes state that the recommended allowance for protein (0.8 g-1▪kg-1▪day-1) is sufficient to meet the requirement of 98% of healthy individuals, and there is a "lack of compelling evidence" to the contrary suggesting that healthy exercising adults do not need additional protein (50a). However, the RDA only seeks to repair losses and prevent deficiency; an "athletically optimal" amount of protein seeks to (1) repair and replace damaged proteins, (2) remodel proteins within muscle, bone, tendon, and ligaments, (3) maintain optimal function of all metabolic pathways using amino acids, (4) support increments in lean mass, (5) support an optimally functioning immune system, (6) support the optimal rate of production of plasma protein, and (7) support other amino acid requiring processes that likely function at rates higher than non-athletes (86).

There is also some evidence for varying levels of efficiency in protein and amino acid turnover based on intake levels (121). While high efficiency can be obtained at optimal levels of protein and amino acid intake, low efficiency and low actual uptake may be associated with protein intakes higher than the optimal level. For an elite athlete seeking to reach and maintain peak condition, efficiency and optimal utilization of protein and amino acids will be key to performing at their highest level.

Nitrogen balance and metabolic tracers have been used to measure muscle metabolic processes, however, each of these methods has potentially confounding factors. While nitrogen balance can be measured over longer time periods, the lack of resolution does not allow optimal protein intakes to be determined. On the other hand, metabolic tracers provide more information about the behavior of muscle metabolic processes but require highly controlled, short-term measurement periods that may not reflect real-world conditions. The recent application of deuterium oxide (2H2O) tracer methodology to measurement of protein synthesis has been shown to accurately measure muscle metabolic processes in detail over longer time periods (42-44, 58). The deuterium method has now been used to measure protein synthesis in both rats (43, 44) and humans (42, 58, 114).

Timing of protein intake has also been investigated in conjunction with exercise, though to a lesser extent than total protein intake. Immediate post-exercise protein ingestion is accepted as an optimal strategy, however, substantial variation exists between these studies as well. Some found no difference between timing strategies (25, 94, 110), while others found greater benefit to pre-exercise ingestion (111) or immediate

post-exercise ingestion (2, 39, 47, 49). To our knowledge, there has been no measurement of interaction effects between timing of protein intake in conjunction with exercise, and total daily protein needs for athletes and active individuals. *It is our central hypothesis that if timing of protein ingestion affects overall net anabolic processes then optimal total protein intake over 24 hours will also change.* Upon completion of this study, it is our expectation that the relation between timing and total protein intake in trained individuals, in an intense, stable training program, will be better understood; such information will provide insight into improving performance in individuals engaged in rigorous sport training.

Objectives, Specific Aims, and Hypotheses

The overall objective of these studies is to determine whether, in well-trained individuals/athletes, total protein requirement is elevated above the RDA and whether immediate post exercise protein intake affects cumulative muscle protein synthesis using deuterium oxide, "heavy water", tracer methodology developed in our laboratory. We also seek to identify any interaction effects between timing of protein intake and total daily protein requirements.

Objectives

The objectives of this study are as follows:

1. Determine whether steady-state trained individuals have elevated total protein requirements

2. Determine whether immediate post exercise protein intake affects muscle protein synthesis and total lean mass

3. Determine whether there is an interaction between timing of protein intake post-exercise and total daily intake

Specific Aims

The specific aim of this study is to determine the effects of varying total daily protein intake and timing of protein ingestion on cumulative muscle protein synthesis responses to high intensity exercise using deuterium oxide (2H2O). We hypothesize that if there is indeed a timing effect, then protein ingestion at a more optimal time will decrease overall protein need. Alternatively, since muscle protein synthesis (MPS) peaks 12-24 hours after exercise, our study is designed to determine whether timing of protein intake matters over the course of 24 hours of recovery following high intensity exercise when total protein is held constant. Total energy will also be held constant in these experiments, recognizing that adequate energy spares protein and sufficient energy is a prerequisite for full activation of protein synthesis signaling pathways. Individuals who have limited energy intake may benefit from such an interaction between timing of protein intake and total protein ingestion. Optimally timing protein intake with exercise could allow such individuals to meet protein needs on limited calories to allow for ingestion of other necessary macronutrients (i.e., carbohydrates for athletes) while consuming enough protein to maintain or grow fat-free mass.

Additionally, our study will address common limitations in previous investigations involving optimal protein intake with exercise. The use of both trained and untrained subjects may have contributed to the variation in recommendations for protein intake. Untrained subjects may have higher protein requirements when

beginning an exercise program, however, there is evidence that rapid adaptations occur to meet this need through an increase in efficiency (18, 60, 102). Thus, trained individuals may require less protein following regular training to maximally stimulate muscle protein synthesis (85), and elevation of MPS may follow a much different time course compared to untrained subjects (14). Shorter experimental periods may not capture adaptations to training or to manipulated levels of protein intake. To minimize effects of training status and reduce effects of adaptation periods, we will utilize trained subjects and longer adaptation periods so that steady-state dynamics can be achieved. Moreover, protein quality and energy intake between groups will be held constant to isolate the total protein and timing effects.

The primary goal of this study is to measure muscle protein synthesis in trained individuals under stable training conditions. It is the athletic community that is most likely to follow diet recommendations, especially in regards to protein intake. While athletes typically ingest high levels of protein, we hypothesize that individuals undergoing consistent training have adapted to their workload and do not have elevated protein needs. This effect may not have been evident in previous studies due to use of untrained subjects, fasted conditions necessitated by metabolic tracers, and/or uncertainty associated with the assumptions made when using previous methodologies. A study that measures muscle metabolic processes in free-living, steady-state trained subjects has not been done before, however, it has the potential to greatly impact the field of sports nutrition.

Hypotheses

The following hypotheses are proposed:

1. Increases in daily protein intake will not have additive effects on cumulative muscle protein synthesis in individuals undergoing consistent training.

2. Cumulative muscle protein synthesis will not be significantly different between immediate and delayed post-exercise protein supplementation.

3. There will be no interaction between total daily protein requirements and timing of protein supplementation post-exercise.

CHAPTER II

LITERATURE REVIEW*

Protein intake requirements to optimize skeletal muscle health have been studied extensively for decades, and although a specific dogma has emerged from this work, considerable controversy still exists. Many studies support the idea that active individuals and athletes have higher protein requirements than do sedentary populations; however, other studies propose that adaptation to intakes near the Recommended Daily Allowance (**RDA**; 0.8 $g^{-1}kg^{-1}d^{-1}$ protein) occurs over time with training through increases in the efficiency and utilization of amino acids and reductions in protein catabolism. Tipton & Witard (110) argued that the question of optimal protein intake is interesting from a scientific point of view, but the applicability of these values to the athletic community is irrelevant due to the excessive intake levels typically seen in athletes. In fact, dietary assessments indicate that intake is beyond even the highest recommendations: 2 to 3 $g^{-1}kg^{-1}d^{-1}$ protein in strength and power athletes, and 1.2 to 1.6 $g^{-1}kg^{-1}d^{-1}$ protein in endurance athletes (110). Although excessive protein intake for athletes may be considered the norm, if athletes are consuming an excess of protein on a limited-energy diet, carbohydrate intake may suffer as a result of elevated protein intake. Because carbohydrate is the key limiting source of energy to fuel muscle contraction during intense exercise, a diet that favors protein over carbohydrates is likely to

compromise glycogen stores and impair both training and performance. In additional, high protein intake may increase the work of the kidneys, negatively affecting hydration status and electrolyte balance, and may also interfere with absorption, contribute to metabolic imbalances, and alter brain neurotransmitter activity (61). Some evidence indicates that efficiency in protein and amino acid turnover varies according to intake levels (18, 102, 110, 121). Although high efficiency may be obtained at optimal levels of protein and amino acid intake, low efficiency and low uptake may be associated with ingestion of protein at higher-than-optimal levels. For athletes seeking to reach and maintain peak condition, efficiency and optimal utilization of protein and amino acids may be key to performing at their highest level.

Many factors account for the controversy in recommendations, including methods of measuring protein utilization, training status of subjects, exercise type and intensity, energy and carbohydrate content of the diet, type and timing of protein intake, and duration of the study. Most studies determining total daily protein requirements in exercising subjects did not account for the timing of protein ingestion, yet other studies using tracer methodologies have shown a strong effect of protein timing on muscle protein synthesis (**MPS**) in response to acute exercise. How to translate acute effects of protein timing on MPS to chronic adaptations with exercise training, and how to determine the ways in which these effects interact with total daily protein requirements, remains largely unresolved. This gap in knowledge is illustrated by Mitchell et al. (77), who found that acute measures of MPS following the initial acute bout of resistance exercise (**RE**) were not correlated with the subsequent muscle gain associated with

chronic resistance exercise training (**RET**). Millward (75) states, "Thus, the key test of adequacy of either protein or amino acid intake must be the long-term response in terms of the specific function of interest" (p. 203). These long-term responses have proven difficult to measure, and it is likely that new methodologies for measuring cumulative MPS (**C-MPS**), such as the deuterium oxide method (**Figure 1**), must be applied to this question in order to better understand the dynamic fluctuations in protein requirements.

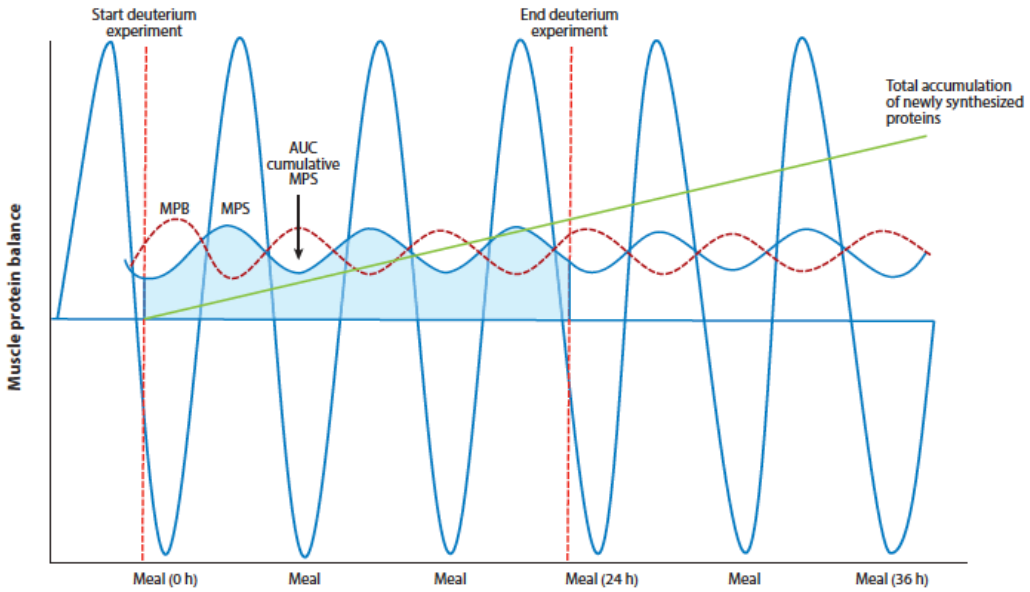

Figure 1 - Muscle protein balance. Overall muscle protein balance is net muscle protein synthesis (MPS) less the muscle protein breakdown (MPB) that is directly related to meals. In an experiment using deuterium oxide to label amino acids entering protein synthesis, the total accumulation of newly synthesized proteins in the time frame of the experiment represents the area under the curve (AUC) cumulative MPS incorporating all fluctuations and influences during that time.

Factors Affecting Protein Synthesis

Blanket recommendations regarding protein intakes should be interpreted with extreme caution because a number of factors can affect the protein requirements of individuals, especially athletes. Factors that should be taken into account include total energy intake and expenditure, exercise history, current training volume and intensity, and the timing of protein intake, all of which will affect muscle metabolic processes and thus alter specific recommendations for individuals (56, 102, 104).

Energy and Carbohydrate Intake

Perhaps the most critical determinant of muscle protein balance is total energy intake, as evidenced by findings that energy intake improves nitrogen balance, even when no protein is consumed (19, 21, 112). Regardless of protein level, insufficient energy results in increased use of protein for energy production, which leads to increased urea nitrogen excretion and negative nitrogen balance (18, 19, 27, 63, 70, 83a, 102). However, even in a hypoenergetic state, RET attenuates and may prevent lean mass losses (87, 89). More specifically, a sufficient supply of carbohydrates has been shown to reduce amino acid oxidation for energy, improve protein balance, and support protein retention through antiproteolytic effects (27, 38, 63, 97, 98). Carbohydrates given alone after RE improve nitrogen balance, whereas carbohydrates in conjunction with protein improve MPS (11, 12, 73, 95, 106, 108, 117). Although high-protein, low-carbohydrate hypoenergetic diets paired with exercise training consistently results in improvements in lean and fat masses (55, 59), such a diet may not be a good strategy for athletes because a decreased carbohydrate intake leads to lower muscle glycogen stores that are critical to

work output during muscle contraction (15, 16). Thus, diets that displace carbohydrates in favor of elevated protein intake may reduce performance and training intensity (65).

Protein Timing

Significant variation exists in the findings of studies examining the timing of nutrient intake and its effect on muscle metabolism. Whereas many studies suggest that timing is important to muscle accretion (3, 7, 39, 50, 62, 64, 109), other studies have found no significant difference between various ingestion times (95, 113). A reduction in training-induced effects was found to occur when protein ingestion was delayed compared with when protein was consumed immediately following exercise (2, 39, 47, 49). Conversely, Tipton & Wolfe (111) propose that MPS is stimulated to a greater extent if amino acids are ingested immediately before exercise, whereas others show no difference in MPS with protein consumption before, one hour after, or three hours after exercise (25, 94, 110). In addition to the various timing intervals used, considerable variation exists in quantities of amino acids or protein consumed during timing studies. The current recommended intake is 20 to 25 g protein (or 8 to 10 g essential amino acids) of intact, high-quality protein (50a), which maximally stimulates MPS with only slight increases in amino acid oxidation (30, 79, 83, 86). The limitations of using tracers and the lack of integration with total daily protein ingestion highlight the need for methods that are able to analyze muscle protein use over the course of days (or longer) and cautions against the extrapolation of short-term analysis of muscle protein metabolism to total daily protein recommendations. As illustrated in **Figure 2**, classic tracer methodologies examine short-term changes in MPS over fixed time frames that

may not capture individual differences in response over longer periods. In contrast, the deuterium method of measuring C-MPS (**Figure 3**) can incorporate many of the hormonal, genetic, activity, and other influences over a more extended period in a free-living, fed state.

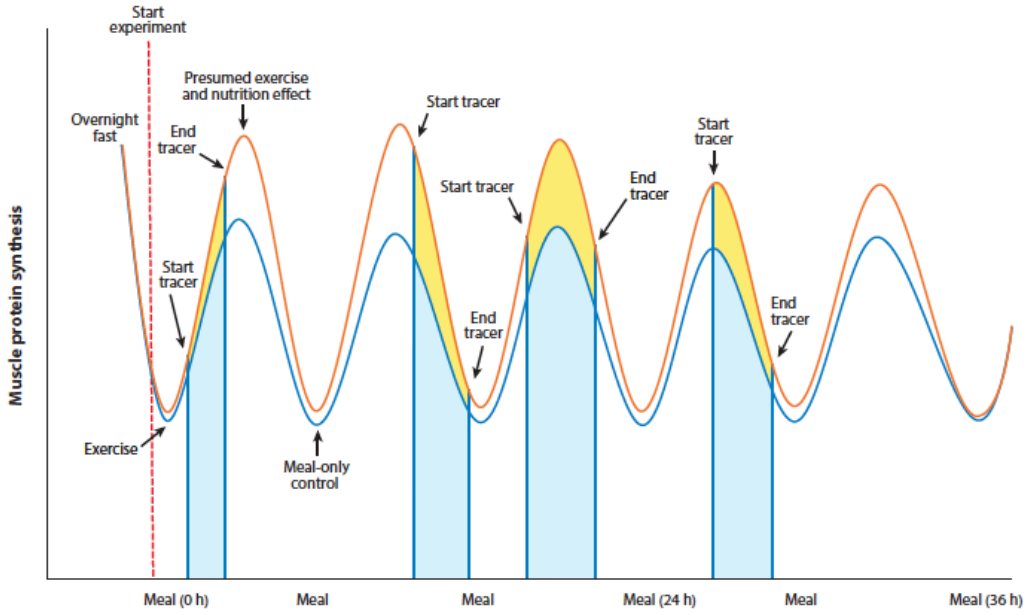

Figure 2 - Classic tracer methodology examining exercise and nutrition effects on muscle protein synthesis (MPS). This methodology requires control of meal consumption because of the diluting effect of the meal on the tracer. Additionally, the tracer infusions are provided at exactly the same time between subjects without regard to interindividual variation in slope and peak MPS (i.e., it assumes the changes in MPS are the same between subjects). This method can be applied to many time points across the recovery from exercise; however, the interindividual variation may be greater the further from the exercise the tracer is applied.

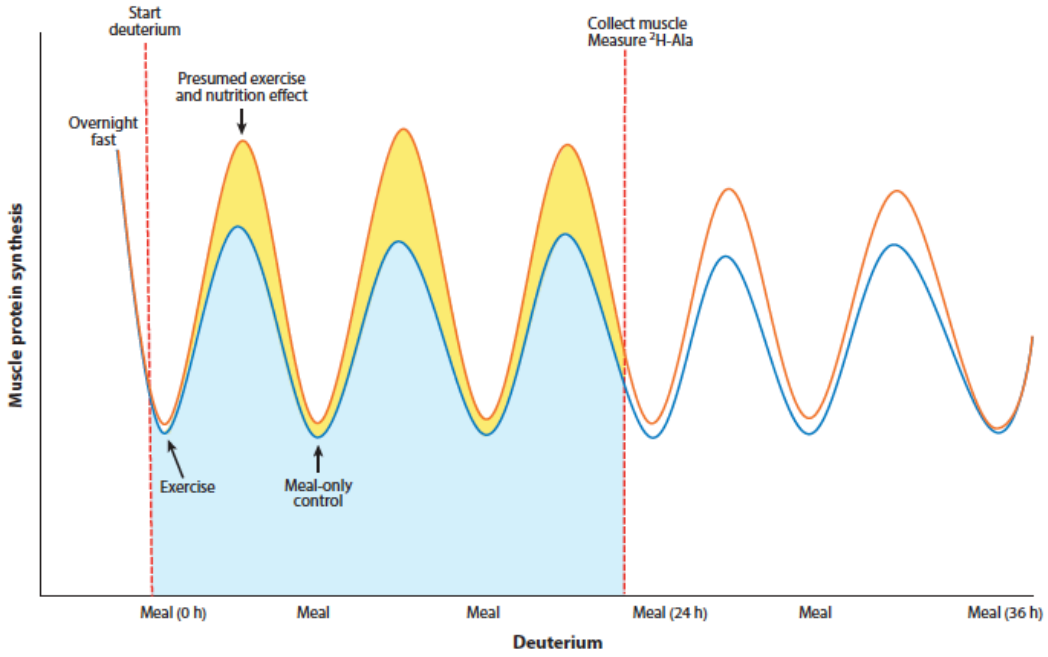

Figure 3 - The deuterium oxide method measures muscle protein synthesis (MPS) and labels amino acids from all sources contributing to the amino acid pool; long periods of measurement can be obtained to determine the cumulative MPS. This method incorporates the effects of all interindividual variations in response times to interventions within the window of measurement. The deuterium oxide method also does not require fasting to prevent diluting the tracer because of the rapid equilibration of the deuterium labeling of the amino acids. Abbreviation: ^{2}H-Ala, deuterium-labeled alanine.

Training Status

Exercise itself may increase MPS and improve nitrogen retention; however, previous training likely plays a role in determining protein requirements (18, 62). Moreover, the changes that occur in MPS during recovery from acute exercise are likely different for trained versus untrained individuals (14). Tang et al. (110) showed that trained individuals exhibit the greatest change in fractional synthesis rate (**FSR**) around 4 hours postexercise and return to baseline quickly, whereas untrained individuals peak around 16 hours postexercise and return to baseline more slowly (54). These and other data are extrapolated into a model of fluctuations in MPS during recovery from exercise in **Figure 4**. Likewise, Murton & Greenhaff (80) suggest that acute transcriptional responses are likely dampened over time with chronic exercise. Highlighting the difference in protein intake requirements between trained and untrained individuals, Lemon (61) estimated that experienced body builders (>3 years) may require 0.9 g/kg per day, whereas novices during the first month of training require approximately 1.5 g/kg per day. Nonetheless, recommendations persist, even by the same author, that endurance and resistance-trained athletes consume 1.2 to 1.4 g/kg per day and 1.6 to 1.8 g/kg per day, respectively, which is above the RDA of 0.8 g/kg per day (62).

The volume and intensity of exercise training may also contribute to protein requirements. Training too frequently leads to decreased gains or increased losses of muscle mass and strength, suggesting that elevated protein intake may be necessary with very frequent training (60). In rats, studies have found that longer or more intense endurance exercise causes a longer period of reduction in the rate of MPS following an

exercise bout (4, 35, 45, 51). Reconciling these findings, Tipton & Witard (110) suggest that the MPS stimulus may be a bell-curve continuum, so that very-low-intensity exercise does not stimulate MPS, increasing exercise intensity increases the MPS response, and very-high-intensity exercise reduces the MPS response. Again, a cautionary approach to generalized recommendations is necessary due to the complex interactions of all factors that affect protein requirements and the short periods of experimental measures that have tested these requirements.

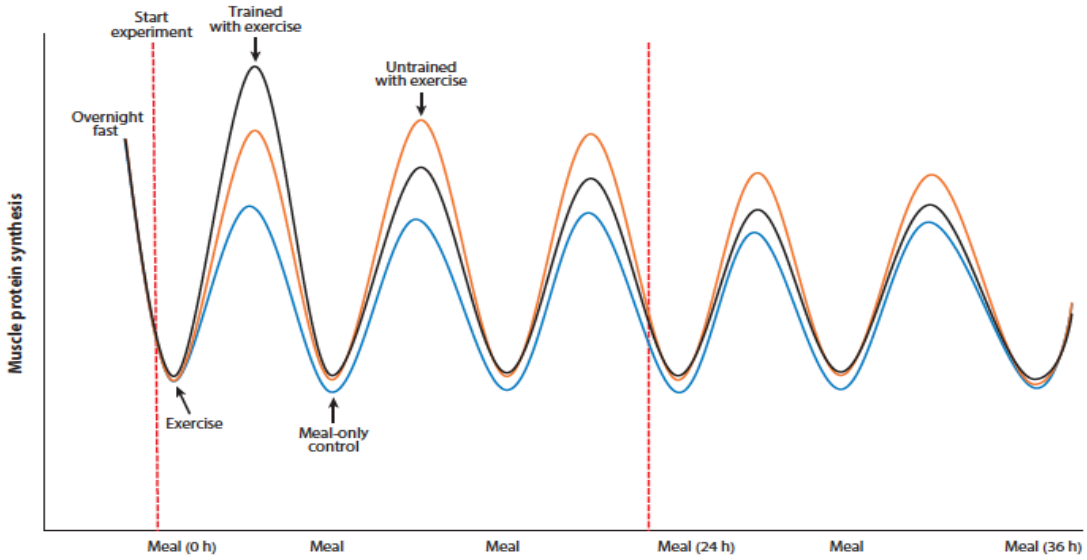

Figure 4 - Change in muscle protein synthesis (MPS) over time in response to exercise and nutrition between trained and untrained subjects (extrapolated from classic tracer methodologies). These studies suggest that trained individuals have a larger, earlier response, whereas the response of untrained subjects is more delayed and sustained. These effects on MPS have not been examined using the deuterium methodology.

Measuring Protein Synthesis

A range of protein recommendations from 0.8 to 2.67 g/kg per day has emerged from many studies (13, 18, 21, 50a, 60--63, 83, 85, 86, 102). Despite the variations in study methods and conclusions, a general integration of the protein requirements has been done several times. Male endurance athletes were estimated to require 1.0 to 1.2 g/kg per day, whereas male strength and power athletes were estimated to require 1.3 to 1.6 g/kg per day (13). Assuming that overall energy needs are met, Lemon (61) estimated that 1.5 to 2.0 g/kg per day from a mixed diet of approximately 12% to 15% energy from protein should be sufficient for strength athletes, although more recently, 1.33 g/kg per day was determined to be a safe intake (85). These estimates of total daily intake are combined with results from tracer studies that recommend 20 to 25 g of high-quality protein immediately following high-intensity exercise, despite the fact that the timing of intake and total intake have never been studied simultaneously.

The evolution of technologies and methods to measure protein accretion suggest that optimal protein intake may be different from current and long-standing accepted norms. Over time, protein requirement studies have used multiple approaches to estimate protein accretion, both indirectly, with nitrogen balance, urinary urea, or N_{τ}-methylhistidine excretion, and more directly, with isotopic tracers. Each approach has its own unique set of strengths and challenges---especially when utilized in exercise studies---which have contributed to the inability to consistently reproduce estimates of optimal protein recommendations, and thus these estimates require interpretive caution.

Additionally, measurements taken during the training adaptation period may indicate an increased protein need, whereas data collected in stable training may show no change or a decrease in need (18). Nonetheless, these small windows of measurement are extrapolated into long-term recommendations under the assumptions that muscle metabolic processes will remain the same and that nitrogen retention stably reflects muscle growth.

The utilization of metabolic tracers faces fundamental issues such as assumptions about the precursor pool of the labeled substance, determination of correct priming dosages, and method of injection/ingestion. The major issues for exercise studies are that assumptions about tracer behavior may not hold when metabolic rates change during exercise (116), as well as uncertainty regarding the interaction between energy requirements of exercise and methodological requirements for fasted subjects. Nevertheless, metabolic tracers are currently the most direct measure of protein metabolism.

Other approaches that show considerable promise have emerged based on the underlying assumptions related to tracer methodologies. One example is the use of deuterium oxide methodologies (**Figure 5**), which overcome numerous hurdles regarding the control of the precursor pool. This model takes advantage of cellular function to label the amino acids in the cell. Thus, whereas other tracer methodologies place paramount importance on controlling the introduction/availability of a fixed tracer with regard to the total amino acid pool, the deuterium approach maintains a reasonably constant tracer pool (relative to the total amino acid pool) because the cell actually labels

its own pool. Thus, eating meals or supplementing with amino acids during the experimental protocol maintains the precursor pool ratio because amino acids entering the free amino acid pool of the cell will be labeled in proportion to the total deuterium availability.

Furthermore, an incredible advantage of this approach is that even amino acids reentering the free pool of the cell from muscle protein degradation are labeled before potentially being recycled into new proteins. The fixed-label methodologies likely underestimate protein synthesis because amino acids entering the free pool from degradation or protein ingestion perturb and/or dilute the overall pool within the cell. Deuterium methodologies dramatically diminish this potential underestimation.

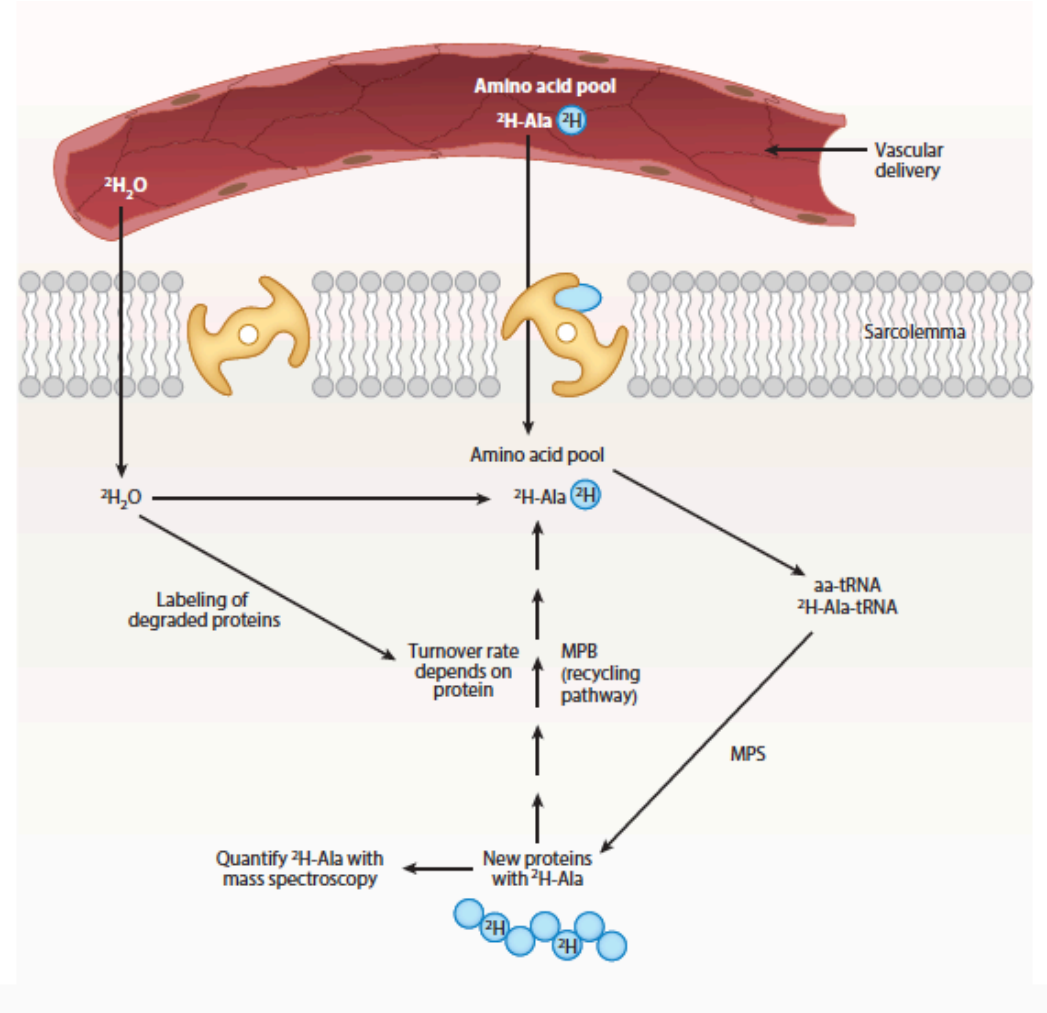

Figure 5 - Incorporating deuterium into newly synthesized proteins. Deuterium equilibrates quickly in vascular, extracellular, and intercellular spaces. Transamination reactions label amino acids in the intracellular amino acid pool provided by the transport of amino acids across the sarcolemma or from turnover of muscle proteins during muscle protein breakdown. Mass spectroscopy quantifies the incorporation of deuterium-labeled alanine (^{2}H-Ala) into newly synthesized proteins. Abbreviations: MPB, muscle protein breakdown; MPS, muscle protein synthesis.

Despite the numerous experimental intervention studies and reviews of previous literature that have called for further studies to measure MPS and muscle protein breakdown (MPB) at multiple time points over a long-term recovery period following chronic exercise training and in conjunction with nutritional supplementation (3, 5, 6, 28, 33, 72, 77, 80), such studies have not been possible to accomplish with fixed-label methodologies. Deuterium methodologies will enable scientists to better capture measures of protein synthesis with dynamic alterations to the precursor pool (as occurs with supplements and/or nutritional intake). These methods also will allow for assessments to occur over longer periods of time and thus permit the direct measure of protein synthesis following interventions, with or without exercise.

Total Protein Intake and Muscle Accretion

Total daily protein intake has been proposed to affect lean body mass. Protein synthesis, protein breakdown, and amino acid recycling efficiency together contribute to lean body mass accretion.

Measured Rates of Protein Synthesis Versus Lean Body Mass Accretion

During the process of determining optimal protein intake, it is often forgotten that MPS is an intermediate measure of lean body mass (**LBM**) accretion. Accretion occurs due to elevated protein synthesis rates relative to rates of breakdown, and it is highly dependent on the availability of amino acids (10, 11, 73, 95, 105). Studies showing increased rates of protein synthesis without a subsequent increase in LBM accretion may suggest that rates of protein degradation are also elevated. However, it should be noted that studies exhibiting elevated rates of synthesis and no change in mass

might be confounded by methodological limitations, such as utilization of fasted subjects who are likely to have suppressed rates of protein synthesis due to insufficient availability of protein or energy. Therefore, the observed increases in protein synthesis in response to energy and/or protein intake may occur only relative to this suppressed state, and simply normalizing rates of synthesis may not be adequate to allow for the accretion of LBM. Furthermore, anabolic responses over time are highly variable and are based on nutrient intake, the energy state of the cell, and whether the muscle is active or quiescent. **Figure 2** illustrates how the magnitude of protein synthesis is dependent upon the time point in which it is measured. Furthermore, interindividual variance may be magnified if the measurement period includes sharp increases in MPS. Although the intervention may acutely affect rates of synthesis, the extrapolation of these highly acute synthesis and oxidation rates over time may not be indicative of C-MPS and LBM accretion.

For example, Biolo et al. (10) showed that amino acid infusion resulted in protein synthesis levels of 150% and >200% in individuals at rest and after RE, respectively. Although the authors hypothesized that amino acid infusion maintains intracellular amino acid concentration so that protein breakdown is attenuated, the observation may only reflect a transition from the fasted to fed state. Because protein synthesis does change on a linear scale of amino acid availability, the 150% increase in MPS may only demonstrate a shift back to baseline values. Indeed, few studies show an actual increase in LBM, which should occur in conjunction with such elevated rates of MPS unless that elevated rate is equally matched by rates of degradation. A meta-analysis by Nissen &

Sharp (82) showed no effect of protein intake on LBM in conjunction with exercise training. Studies of older men and women performing RET showed no difference in strength, protein accretion, fat mass, or lean mass between low- and high-protein-intake groups (3, 34, 53), suggesting that acute measures of protein synthesis using some classic tracer methodological approaches may overstate the impact of nutrient intake on muscle growth.

In addition, the extrapolation of nitrogen balance to LBM accretion may not be valid, as illustrated by the results of Tarnopolsky et al. (104), where the positive nitrogen balance observed would predict the bodybuilders in the study to have gained 300 to 500 g of LBM per day, which did not occur. Indeed, this same study showed highly trained bodybuilders to have only 12% greater protein requirements than sedentary controls, highlighting the disparity between nitrogen balance and LBM accretion as well as the difference between the protein requirements of trained and untrained individuals.

It is generally expected that chronic training results in LBM accumulation but that the rate of accumulation diminishes over time as one becomes more trained. With regard to nutritional impact, Wolfe (117) proposed that the plateau in net muscle anabolism observed as a result of RET may necessitate greater protein/amino acid intakes to elicit the same magnitude of anabolic effects observed after acute bouts of exercise in untrained individuals, supporting the idea of high protein intakes even in the trained state. However, higher levels of protein intake in strength-trained individuals caused a nutritional overload such that additional protein above moderate levels resulted only in an increase in leucine oxidation, with no observed gains in LBM (103).

Untrained individuals often see higher levels of LBM accretion with exercise and protein/amino acid interventions, whereas trained individuals do not, despite progressive overload [as discussed by Atherton et al. (6)]. In fact, a recent study showed no correlation between acute measures of MPS and changes in leg volume following chronic RET, highlighting the changes that occur in the acute MPS response as an individual progresses from untrained to trained (77). Atherton et al. (6) suggest that the deuterium method will allow for the determination of the time course of hypertrophy and its relationship with MPS, which would clarify the anabolic effect of protein ingestion when moving from the untrained to the trained state.

Although RET significantly increases strength and LBM, the addition of protein supplementation immediately after RET in seniors showed that variability in protein intake is not associated with LBM accretion; groups that were high versus low in total daily protein intake showed similar increases in LBM with 12 weeks of RET (3). Meredith et al. (70) found that an additional energy supplement resulted in greater hypertrophy, suggesting that total energy may be an important predictor of muscle protein accretion.

Protein Synthesis Versus Breakdown

Cumulative muscle protein synthesis is determined by the long-term balance between protein synthesis and protein breakdown. Additionally, amino acid recycling efficiency can play a role in determining overall protein balance.

Cumulative Muscle Protein Synthesis

LBM accretion can result only from chronic positive net protein balance, and such C-MPS is the sum of the synthetic responses to each exercise bout. As highlighted by Murton & Greenhaff (80), the contributions of MPS and MPB to overall improvement in LBM and strength in conjunction with exercise training is still largely unknown owing to the lack of long-term studies. It is commonly accepted that exercise usually causes an increase in MPB, which must be later offset by a greater increase in MPS in order for LBM accretion to occur. In fact, participating in a single session of RE in a fasted and/or untrained state results in MPB that exceeds synthesis (88). RE also stimulates MPS between 40% and 150% above resting levels (9, 88, 89), but muscle protein balance remains negative in the fasted state postexercise due to the increase in MPB. However, MPB has been shown to change less than MPS does in response to nutrient intake and exercise, and thus MPB may have less overall effect on LBM accretion (57). MPS is increased following exercise, though this response may not be stimulated for some time (48). Surplus protein/amino acids during digestion have thus been proposed to aid protein synthesis and/or minimize protein breakdown, allowing a net positive protein balance and LBM accretion (61). Therefore, the cumulative synthetic response over the 24 hours following exercise and the long-term response to chronic training are the primary measures of interest, but few studies exist using these approaches.

As noted previously, the extrapolation of acute protein synthetic rates may not be sufficient to predict the response to chronic exercise training. Studies measuring MPS

responses to the same nutrition and exercise intervention found no correlation between MPS following the first acute bout of exercise and muscle hypertrophy after the training period (68, 77). Therefore, although synthetic responses between acute and chronic exercise bouts may be qualitatively similar, training status determines the magnitude and time course of these responses. Consistent with that notion, studies have shown that MPS increases in most untrained individuals postexercise, whereas a dampened response is observed in trained individuals (9, 88, 100, 107). It is important to note that these measures assessed acute time points soon after the exercise period (as described in **Figure 2**), making it difficult to interpret whether anabolism no longer responds to an acute bout of exercise with training or whether an "optimal" window for anabolism has shifted to another period. Thus, the cumulative synthetic response may predict LBM accretion, but acute responses within the few hours following a single exercise bout may not accurately predict accretion.

Measures of Muscle Protein Breakdown

The measurement of overall muscle protein accretion is based on the relative contributions of protein synthesis and degradation. From a methodological standpoint, the assessment of MPB is fraught with assumptions, many of which are reliant on the assessment of protein synthesis and LBM to interpolate rates of degradation. Other studies have used specific markers that arise from degraded muscle proteins or assessed the appearance of amino acids in blood/extracellular compartments as an indication of the catabolic state of the muscle. For example, in early studies utilizing indirect measures of muscle protein metabolism, such as urea excretion or 3-methylhistidine (3-

MeHis), some studies indicated an increase (35, 96), no change (26, 90), or a decrease (81) in MPB with exercise. Given the presumed changes in amino acid recycling with exercise training, it is very difficult to determine if the altered mass with exercise training is actually due to an altered rate of degradation or synthesis, or whether the ability to capture the label in the cytosol (or blood) has been affected by the capacity for that label to be reincorporated into skeletal muscle protein through recycling.

Some investigators have speculated that the increase in MPB is confined to the sarcoplasmic pool to provide amino acids for synthesis of myofibrillar proteins. This idea is based on the observation that the sarcoplasmic protein fraction turns over at a rate that is twofold greater than that of myofibrillar proteins in the fasted state (30, 78). Although that is true, it should also be noted that the capacity to actually measure changes in rates of protein degradation is enhanced in faster turnover pools (cytosolic) when compared to slower turnover pools, particularly when changes are acutely assessed over a relatively short window. Thus, it is difficult to determine if the altered rates of degradation are mostly limited to sarcoplasmic proteins with exercise training or whether changes in rates of degradation occurring in the myofibrillar fraction are not reliably measured over the brief period they are assessed. A reliable, direct measure of MPB currently does not exist. Likewise, no single measure of protein degradation can account for how many times an amino acid may reenter the anabolic apparatus, rendering the appearance of that label in the blood or urine as a predictor of protein breakdown less interpretable. For example, a study utilizing a nonradioactive technique involving puromycin incorporation into peptide chains found differences in MPS rates in different

muscle fiber types (46). Little difference was found between type 2a and type 1 muscle fibers; however, types 2b and 2x had significantly lower rates of MPS than did type 2a (46). Miller et al. (72) developed a mathematical model from published data and coupled it with an in vitro experiment to show that a four-week period was necessary to measure all protein synthesis in mixed tissue. They found that proteins that turn over more rapidly will increase the label incorporation in a sample, creating a bias toward these proteins and violating the homogeneous protein pool assumption of stable amino acid isotopes (72). This model also verified that the prolonged labeling period allowed by deuterium methods has a greater sensitivity to slower turnover proteins and proteins in lower abundance in skeletal muscle (72). Establishing reliable estimates of longer-term MPS, as made possible by deuterium methods, will allow more systematic and accurate determination of average synthetic rates in all proteins of skeletal muscle. Furthermore, as noted previously, use of this methodology allows for the measure of MPS under a variety of physiological stimuli, including alterations of recycling efficiency. Ultimately, deuterium methodologies positively impact the interpretive value of MPS measurements.

Efficiency of recycling

As discussed previously, many have proposed that chronic training results in adaptation to protein intakes near the RDA by increasing efficiency and utilization of amino acids. However, methods such as nitrogen balance and traditional metabolic tracers have been unable to capture such changes in recycling efficiency. Nitrogen balance fails to account for increased efficiency of nitrogen use at marginal protein intakes, and thus results would favor using higher-protein diets. Likewise, radioactive amino acid tracers cannot

capture changes of protein synthesis, particularly over extended periods, with alterations of recycling efficiency. Using such tracers does not allow determination of how many times a labeled amino acid was involved in the formation of nascent proteins because they were captured and/or recycled from short-lived, recycled proteins (**Figure 5**). As noted previously, a distinct advantage of deuterium methodologies is that amino acids arising from degraded proteins are labeled by the cell once they enter the free pool. It is therefore impossible at this time to determine whether exercise training actually results in a lost anabolic response to the acute bout of exercise or if the improved recycling from degraded proteins diminishes our capacity to measure anabolic responses. Either of those outcomes could negate a need for elevated protein intake with exercise training (albeit for very different reasons). It should be noted that if muscle protein turnover increases without an increase in recycling efficiency, it is doubtful that one could sustain an elevated level of LBM with a diminished protein intake.

Alternatively, some evidence suggests that intake levels are the basis of variations in protein and amino acid turnover efficiency (121). Whereas high efficiency can be obtained at optimal levels of protein and amino acid intake, low efficiency and moderate actual uptake may be associated with elevated protein intakes. Campbell et al. (23) compared low (0.8 g/kg per day) and high (1.62 g/kg per day) protein intakes over 12 weeks of RET in untrained men and women. The low-protein group showed increased efficiency (measured as greater nitrogen retention) and rates of protein synthesis, whereas high protein intake was associated with increased leucine oxidation. Moreover, very low protein intakes (<0.8 g/kg per day) may be associated with very

high efficiency but compromised metabolic function, indicating nutrient insufficiency. Nitrogen balance methodologies can be misleading because such marginal protein intakes can yield net balance either through increased efficiency or downregulation of processes requiring protein/amino acid (120). Although radioactive amino acid tracers may be able to measure overall MPS to determine whether downregulation has occurred, direct measurement of the efficiency of amino acid is thus far not possible.

Individuals in training, especially athletes, are concerned with maximizing efficiency and optimizing nutrient intake. However, such individuals commonly attempt to accommodate muscle metabolic processes by overfeeding protein and amino acids in an effort to avoid the downregulation of processes requiring amino acid. Such behavior assumes that these individuals expect to elicit a greater anabolic stimulus by engaging in regular training, though this may not be the case. It is unclear whether additional nutritional protein is required to maintain the accrued muscle mass, particularly given the elevation of metabolic rate. If the adaptations to exercise training include an increased efficiency of amino acid uptake and recycling, it is indeed possible that increased overall protein requirements in the diet to support this heightened metabolic turnover may not be as necessary as once believed. Campbell et al. (24) recognized such adaptation as a desirable response, defining it as "metabolic changes that occur in response to changes in protein intake and result in the establishment of a new steady state without a compromise or loss in physiological function" (p. M376). In comparison, the process of accommodation is defined as a survival response and "refers to further metabolic changes in response to the decreased protein intake that the body undergoes to

establish steady state, but only with a compromise or loss in physiological function" (24, p. M376). Without direct, accurate measures of efficiency and recycling, it is difficult to tell whether the "new steady state" anabolic outcome is because of an actual change in the requirement for maintained protein homeostasis or because of our inability to measure altered anabolic responses in the presence of exercise-induced improvements of amino acid recycling. The ability of deuterium to label amino acids coming from this recycling pathway (**Figure 5**) highlights an important strength of this method to measure MPS.

Timing of Protein Intake and Muscle Protein Synthesis

Timing of protein supplementation in conjunction with exercise has been proposed to affect lean body mass. Protein synthesis, protein breakdown, and amino acid recycling efficiency together contribute to lean body mass accretion. Various supplementation timing strategies have been suggested to affect these measures.

Measured Rates of Protein Synthesis Versus Lean Body Mass Accretion

Many studies have attempted to determine a supplementation strategy to elicit an optimal anabolic response in conjunction with individual exercise bouts, and many have shown the timing of nutrient intake to be important to muscle accretion (7, 64, 111). Acute studies show that muscle is sensitive to ingestion of nutrients, especially amino acids, for up to three hours following RE (10). Such studies have consisted of short-term measurements of muscle metabolism and extrapolation of the cross-sectional measure of increased MPS (**Figure 2**) to the potential for increased overall accretion. However, as discussed in regard to total protein intake, the extrapolation of acute measurements may

not in fact reflect the cumulative protein synthesis in response to exercise that results in LBM accretion.

Although some controversy exists regarding whether preexercise supplementation is preferable to postexercise supplementation, it is generally agreed that postexercise protein supplementation yields the greatest increase in MPS. Tipton & Wolfe (111) propose that the increase in MPS is smaller when amino acids are ingested immediately after exercise, as opposed to immediately before, as a result of enhanced amino acid delivery to muscle due to exercise hyperemia. Campbell (22) states that the greatest anabolic state is achieved soon after an individual who has consumed protein performs RE. It is important to note that those studies, as well as other studies exploring preexercise protein supplementation effects, were largely conducted during a period of net negative nitrogen balance, when FSR may actually be suppressed. Because MPS is likely downregulated during exercise of moderate to high intensities and for some time afterward due to the dedication of adenosine triphosphate to work output, administration of amino acids either immediately prior to or shortly after exercise may take advantage of exercise-induced hyperemia to deliver both the nutrient and the tracer to the muscle, but it is questionable whether such a dampened MPS response will actually allow the nutrient and/or tracer to be incorporated into new tissue. Thus, the controversy behind the timing of protein should not overshadow the importance of the methodological conditions through which these measures of MPS were obtained.

A review of past literature does not yield unequivocal evidence for any one timing strategy for protein intake that can be positively correlated with changes in LBM

with exercise, despite the common recommendation to consume protein immediately postexercise (5). Some studies presented no difference in MPS with protein consumption before, one hour after, or three hours after exercise (25, 94, 110); however, other studies showed that delaying protein ingestion by two hours in both young and older individuals caused a reduction in training-induced effects compared with immediate protein consumption (39, 47). Esmarck et al. (39) reported increases in LBM and muscle hypertrophy in older men taking a 10-g protein supplement immediately after exercise over a 12-week period, whereas no significant changes were shown in the group consuming the supplement two hours postexercise. Other longitudinal studies confirmed this finding, showing greater increases in strength and muscle mass with consumption of essential amino acids immediately postexercise (2, 49). It is important to note, however, that the fasted state causes an increase in MPB preexercise and the persistence of net negative amino acid balance into the postexercise period. Such a scenario could account for significant differences between subjects fed immediately postexercise and control or delayed-intake subjects who inherently have an increased period of MPB due to methodological requirements as opposed to intervention effects. One study showed that a group that consumed a protein and creatine supplement immediately before and after exercise had greater increases in LBM, strength, and type II fiber area than the group that ingested the same supplement at different times over the course of the day (29), although the specific cause of these positive effects cannot be elucidated because both protein and creatine were used. Another study found no effect of protein supplementation immediately before and after exercise on increases in skeletal muscle

mass and strength after three months of RET in elderly men habitually consuming adequate dietary protein of approximately 0.9 g/kg per day (113). However, evidence suggests that elderly subjects may have a decreased anabolic response to protein consumption and thus may realize benefit only from an elevated protein intake (28). A crossover study by Tipton et al. (106) measured MPS in three male and three female subjects following acute RE and ingestion of either an amino acid or placebo solution and found that muscle protein balance is negative after exercise in the postabsorptive condition without amino acid ingestion, but positive (anabolic state) if amino acids were ingested during the 225 minutes immediately following exercise. As mentioned above, the tracer methodology used requires fasting exercise to be performed, which may explain such results. It is also possible that the timing of other nutrients affects protein needs (62). For example, ingestion of carbohydrates following exercise can enhance muscle glycogen resynthesis and, together with protein ingestion, stimulate MPS. One study found that 100 g of carbohydrates administered one hour after RE improved muscle protein balance so that it was not different from zero, but positive balance could not be achieved (12). Carbohydrates are protein sparing due to the antiproteolytic effect of insulin, and thus carbohydrate consumption may prevent MPB but cannot support LBM accretion, as reviewed by Aragon & Schoenfeld (5).

In addition, the time course of elevated MPS may differ between trained and untrained subjects (14). Tang et al. (101) showed that trained individuals exhibit the greatest change in FSR around at approximately four hours postexercise, with FSR returning to baseline quickly. FSR in untrained individuals, however, peaks around 16

hours postexercise and returns to baseline more slowly (54). Damas et al. (32) compiled data from multiple studies (54, 67, 88, 100, 101, 118, 119) to characterize the acute response of trained and untrained individuals to a bout of RE (see **Figure 6**). Those collective data demonstrated a transient synthetic response in trained individuals, whereas the synthetic response to the exercise bout in untrained individuals is slower and remains elevated over an extended period. However, it is important to keep in mind the previously discussed methodological limitations of the studies whose data are used in this analysis. Although **Figure 6** appears to show the time course of MPS, each data point actually represents an acute measurement (in contrast to C-MPS measures, as illustrated in **Figures 3** and **4**), making their area under the curve misleading. As such, the conclusions drawn from this figure should be regarded with caution. Based on this figure and other data, we hypothesize that free-living individuals respond to training and feeding as illustrated in **Figure 4**, with variable responses between trained and untrained subjects. Also, because turnover rates may be faster or slower depending on the specific proteins in the muscle, it may be hypothesized that in comparison with untrained subjects, trained athletes are more responsive to rapid turnover proteins. Evidence exists to support the theory that the acute response to exercise in untrained individuals becomes more specialized with training (36, 115). Thus, it has been generally concluded that MPS is greater in the untrained state than in the trained state, highlighting the increased capacity for LBM accretion in untrained individuals. Indeed, a comparison of untrained and trained subjects found the untrained group to have significantly greater hypertrophy after 21 weeks of RET even though the trained group performed a higher total volume of

work (1). Thus, the acute synthetic responses to exercise may differ significantly between trained and untrained individuals such that extrapolation of these measurements likely is not an accurate measure of these individuals' potential for LBM accretion. Multiple studies have found little or no correlation between initial acute responses to exercise and subsequent hypertrophy following training (6, 77, 84). One study used principal components analysis to show that pretraining acute anabolic signals did not share variance with LBM gains, though a weak relationship was observed with postexercise anabolic signaling (84); this result suggests that phosphorylation may be involved in acute remodeling leading to hypertrophy but remains a weak predictor of LBM accretion in response to chronic exercise. Wilkinson et al. (115) showed that mixed MPS may decrease with chronic resistance training, but the change is due to a decrease in mitochondrial MPS without a decrease in myofibrillar MPS. Additionally, they propose that with training, a state of "signaling efficiency" is reached, wherein key anabolic signaling molecules are able to be activated and deactivated more rapidly than in the untrained state. Generally, studies with untrained subjects have found benefits to timing protein intake with exercise, suggesting that the acute responses associated with initial exposure to exercise may require special accommodations in terms of timing nutrient intake (5).

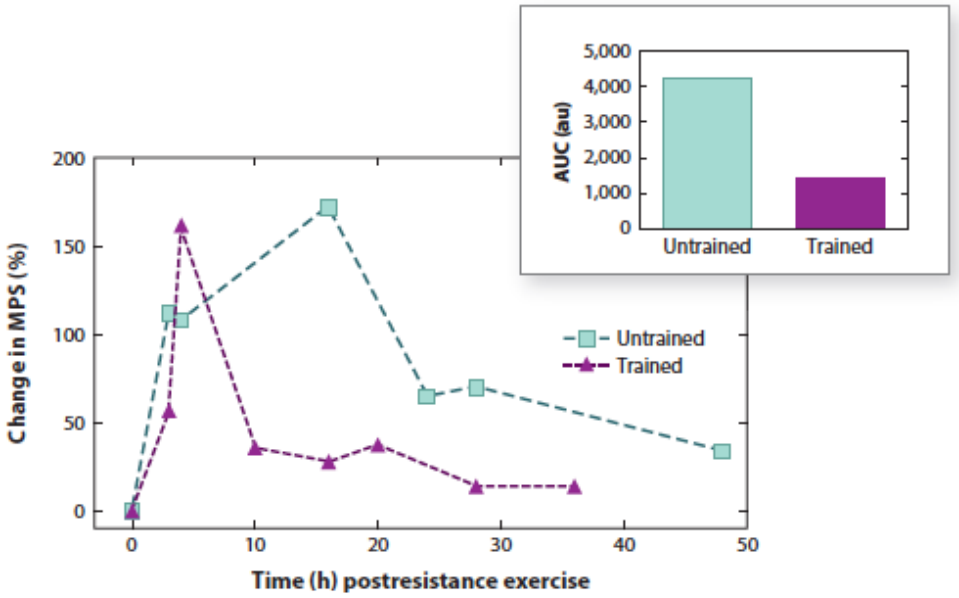

Figure 6 - Projected time course of muscle protein synthesis (MPS) in humans, compiled from several investigations. Figure used with permission from Reference 32. Abbreviation: AUC, area under the curve.

It has long been hypothesized that a single bout of high-intensity work can lead to a robust anabolic response for up to 48 hours, even without feeding. Indeed, a recent study found that both mitochondrial and myofibrillar rates of protein synthesis were elevated for up to 28 hours following an acute bout of high-intensity exercise, and both were significantly higher at 24 to 28 hours compared with 0.5 to 4.5 hours postexercise (33). This phenomenon has been referred to as the window of anabolic opportunity, and it is hypothesized that feeding during this period should stimulate a greater response than feeding at rest. As such, any meal containing an amino acid source consumed within 24 hours of exercise should increase MPS. It is necessary, then, to measure metabolic

processes over longer periods to accurately determine the effects of supplementation timing. Such quantification has not been possible using traditional methodologies for measuring MPS and MPB. Acute measures of MPS in response to exercise and nutrition interventions have led to the current dogma that protein should be ingested immediately, or at least within two hours, following exercise bouts. Hartman et al. (47) suggest that acute changes in protein turnover during postexercise recovery can be at least qualitatively predictive of chronic adaptations to different training or feeding interventions. However, because there has been no true quantification of C-MPS over extended time periods, this idea remains speculative.

Protein Synthesis Versus Breakdown

C-MPS is not dependent on individual fluctuations over the time course following exercise bouts but rather the end summation of synthetic responses (**Figures 1** and **3**). Methodological approaches that allow for the continued assessment of protein synthesis over time must be robust enough to measure rates of synthesis even when they are impacted by protein breakdown or nutrient intake. Our understanding of protein turnover using previous methodological approaches is that an exercise bout results in a net loss of muscle protein due to decreased or unchanged protein synthesis and increased protein breakdown (20, 26, 96). As noted previously, evidence suggests that the mechanism for the greater exercise-induced effects observed when amino acids are supplemented immediately postexercise involves an attenuated MPB response (10, 106), which would normally increase following exercise. As Burd et al. (14) state, the "methodological shortcomings for measuring fractional breakdown rate, a direct

measure of muscle protein breakdown, in skeletal muscle largely precludes its ability to be utilized in the fed state and therefore is leaving an incomplete understanding of muscle protein turnover following exercise" (p. 1698). Determining the long-term time course of muscle metabolic processes over 24 to 48 hours postexercise is critical to the understanding of MPB and MPS and how they contribute to overall C-MPS.

Integration of Recommendations for Timing of and Total Protein Intake to Optimize Muscle Protein Synthesis and Accretion with Resistance Exercise Training

While total daily protein intake and timing of protein supplementation with exercise have been proposed to affect lean body mass, no interaction effects between the total consumption and timing have been investigated. Potential mechanisms for interaction exist and could be tested in future studies using the deuterium oxide labeling methodology.

Potential Mechanisms for Interaction

Much work has been done to establish recommendations for timing of and total protein intake for exercising individuals. The interaction of these two factors, however, has been largely ignored. If, in fact, timing of protein intake can be optimized, then the increased uptake during this period may allow an overall reduction in the total amount of protein consumed over the course of the day. In other words, if MPS is highest following exercise, then supplementing in conjunction with the exercise-induced anabolic window should provide an adequate supply of amino acids to the muscle precisely at the time they are needed for synthesis. Thus, protein needs over the course of the day may be

only marginally increased above the normal recommended intake. Such reasoning assumes that the acute muscle protein synthetic response immediately following exercise is indeed the optimal time point for protein supplementation. Two alternatives exist to this hypothesis. First, if immediate postexercise protein is valuable, then it is possible that the total protein intake necessary to optimize muscle responses is higher than previously suggested, because studies of total protein intake never accounted for timing and therefore may have limited the potential of the response. Second, if timing doesn't matter and optimal total protein over the entire recovery period is the key factor in muscle responses, then results of deuterium-measured C-MPS would show no difference when total protein is held constant and timing is varied.

Although most studies suggest that a greater anabolic state requires a greater total protein intake, Andrews et al. (3) show, at least in elderly adults, no difference in LBM accumulation with RET across a range of total protein intakes when a supplement is provided immediately after exercise. They argue, "Consuming protein in the post-exercise period may be a way to minimize overall protein intake and conserve fat-free mass during periods of either increased energy expenditure or marginal protein intake" (3, p. 369). Their argument highlights the need for methods that are able to analyze muscle protein metabolism over the course of days or weeks as well as cautions against the extrapolation of short-term analyses of muscle protein metabolism to total daily protein recommendations.

Deuterium Methodology

The deuterium oxide methodology for measuring muscle protein synthesis could alleviate confounding factors associated with current methods of measuring muscle protein synthesis.

Deuterium

Issues with metabolic tracers include the necessity for fasted and resting subjects, the need for adequate time to achieve a steady state of enrichment, and the uncertainty regarding the size of the precursor pool. Even when accurately assessed and controlled, the limited duration of these measures makes interpretations about anabolic responses problematic for anything other than proteins with short half-lives. It is important to note that ingestion of nutrients will dilute tracers and change precursor labeling assumptions. In recent years, interest has grown in a methodology that allows measurements of MPS over longer periods in free-living subjects. The advantage of using deuterium oxide is that it rapidly equilibrates with the total body water pool in approximately two hours, which allows for rapid access to hydrogen-containing substrates. As such, deuterium molecules are incorporated into multiple metabolic pools and tissues because they can be exchanged with existing hydrogens during metabolic processes (**Figure 5**), and this exchange occurs in proportion to the deuterium pool as it relates to the total body water pool. By taking advantage of this deuterium labeling process, this molecule has been successfully integrated into protein synthesis measurements in animals (8, 17, 31, 37, 43, 44, 52, 71, 122) and humans (42, 58, 66, 92, 99). Because deuterium oxide has ubiquitous access to water, deuterium levels in the

organism can be easily controlled, maximizing the stability of the precursor pool over the period of measurement.

A study by Wilkinson et al. (114) utilized deuterium over eight days of RET to measure myofibrillar, sarcoplasmic, and collagen protein fractions in eight untrained, healthy young men. Subjects undertook one-legged RE in order to confirm work by others as to the capacity of deuterium oxide methodologies to measure MPS. No changes were detected in the nonexercised leg; however, myofibrillar protein synthesis was significantly greater in the exercised leg, with collagen protein synthesis tending to increase, whereas no change was observed in the sarcoplasmic fraction. Another study compared the FSR measured by both deuterium and phenylalanine flooding in rats following RE (44). Neither method detected an effect of RE on FSR in mixed gastrocnemius, plantaris, or soleus muscle, and the actual percentage of newly synthesized proteins was greater in the measurement taken at 24 hours than at 4 hours for both deuterium and phenylalanine. We can conclude from deuterium oxide studies that this method provides results that are qualitatively similar to prior approaches but may be quantitatively different.

Future Directions

Much work has been done to develop the current total protein recommendations and to determine the factors that affect overall protein needs, but the timing of protein intake is one factor that has been largely ignored. Although the timing of intake and its stimulation of MPS have been studied independently, no studies exist related to its effect on total protein needs of the athlete. With acute exercise, timing of protein intake has

been shown to affect MPS. However, studies have not yet examined total protein intake needs over an extended recovery period when timing is optimized in conjunction with repeated exercise bouts. If the timing of protein intake does indeed stimulate MPS to a greater degree, then optimal timing may decrease total daily protein needs. Thus, individuals who have limited energy intake could benefit from such an interaction between timing of protein intake and total protein ingestion. Optimally timing protein intake with exercise could allow such individuals to meet protein needs on limited calories to allow for ingestion of other necessary macronutrients (e.g., carbohydrates for athletes) while consuming enough protein to maintain or increase LBM. Alternatively, if total protein needs are not altered, the importance of nutrient timing may be overstated within the context of the entire recovery period, and only total protein consumption should be monitored. Given that MPS peaks 12 to 24 hours after exercise (or even after 48 hours in untrained individuals), future studies should be designed to determine whether the timing of protein intake matters over the course of 24 hours of recovery following high-intensity exercise when total protein and energy are held constant. Deuterium methodology may be useful to investigate the relationship between timing and total protein intake in trained individuals undergoing an intense, stable training protocol, and ultimately to rectify two parallel lines of research on protein intake and exercise.

Another goal of future studies should be measurement of MPS across training states to determine important transitions in anabolic state with exercise. Untrained subjects beginning an exercise program may have higher protein requirements, but the

time course of increased protein need and the effect on efficiency is unknown (18, 60, 102). Evidence suggests that trained individuals require less protein following training to support the maximal protein synthetic response (85) and may exhibit a different time course of elevated MPS (14). Shorter experimental periods may not capture adaptations to training or to manipulated levels of protein intake. To minimize the effects of training status and reduce the effects of adaptation periods, future studies should use trained subjects---who are more likely to consume high-protein diets---and longer adaptation periods so that steady-state dynamics can be achieved.

The development of new methodologies together with the lack of information on the interactions between total protein intake and timing of intake highlight the need for new studies. The limitations of previous methodologies have left gaps in our understanding of C-MPS in free-living individuals. Damas et al. (32) state that to determine the potential for protein accretion after a bout of RE, analyses of the time course and the overall response of MPS are critical. In addition, because multiple factors interact to affect synthesis and breakdown following exercise, a methodology is needed that can measure these processes in free-living individuals. C-MPS and LBM accretion in an individual represent an integrated response of the sum of these factors, which include RE bouts, nutrition, sleep, general activity, and genetic predispositions. Focusing on any one without regard for the others leaves an incomplete picture of muscle metabolism and limits the applicability of results to free-living subjects.

Damas et al. (32) also pointed out the current lack of information on muscle metabolic processes in the days and weeks (as opposed to hours) following RE. C-MPS

is not only a summation of multiple factors, as listed above, but also a summation of the muscle metabolic processes over time. A complete characterization of muscle plasticity, growth, and remodeling in response to RET has yet to be realized. Measurements of muscle metabolic processes over longer time windows would allow a better understanding of the effects of RE and RET on C-MPS and subsequent LBM accretion. Indeed, Garlick et al. (41) suggest, "Studies with more sensitive methods over longer periods of dietary treatment may be necessary to detect slow rates of accumulation of protein of 1 gN/d, which is equivalent to only 0.05% of total body N per day" (p. S41). In other words, small changes in protein accumulation may not be seen when measured acutely but could be important to LBM accretion over time. Thus, the effects of the interaction of total protein intake and timing of intake may be seen only if muscle metabolism can be measured over longer time frames following exercise.

Summary

Although the field of sports nutrition currently accepts that a protein intake up to 1.8 g/kg per day is recommended for highly active individuals (50a), and protein/amino acid supplementation is critical during the postexercise anabolic window (5), a great deal of uncertainty exists regarding these recommendations. Previously, nitrogen balance methods have been used to measure muscle metabolic processes over extended periods of time but with low resolution. Metabolic tracers have allowed these processes to be studied in greater detail but only for short periods of time and under fasted conditions. A method utilizing deuterium oxide (^2H_2O) has also been identified to enable the measurement of muscle metabolic processes with a great deal of accuracy over extended

periods of time. This approach warrants further study to examine total protein intake in the context of timing of protein intake, which has not been methodologically possible to accomplish until now.

As mentioned above, deuterium oxide methods have been developed to address the issues inherent in traditional methods, such as nitrogen balance and metabolic tracers. Although the use of nitrogen balance is adequate for establishing requirements to prevent deficiency, it is not likely to be adequate to determine optimal intake for maximizing muscle metabolic processes. Metabolic tracers are currently the most direct measure of protein metabolism; however, this method faces critical issues concerning the priming dosage, the method of injection/ingestion, assumptions about precursor pools, and assumptions about tracer behavior. A major issue with metabolic tracer use in exercise studies is that assumptions about tracer behavior may not hold when metabolic rate changes (60, 116). Utilization of deuterium methods will circumvent these limitations in order to more accurately measure MPS. Gasier et al. (44) concluded that metabolic tracer and deuterium methods provide qualitatively similar results but may be quantitatively different. Deuterium methods may prove to be better in establishing working recommendations because subjects are not required to be fasting or resting, as they must be for metabolic tracer studies. This allows subjects to be free living, which removes potentially altered metabolic processes that occur during a fasted state. Tracer flooding may also increase pool size and alter metabolic processes, interfering with a true measurement of the response to an intervention. Deuterium methods, in contrast, allow the cell to do its own labeling of amino acids so that the precursor pool is easily

maintained by sustaining overall deuterium oxide levels in the organism (40, 44, 58). By utilizing this novel methodological application, future work will be able to more accurately measure MPS in free-living subjects to better replicate real-world scenarios.

Although athletes typically ingest high levels of protein, we hypothesize that individuals undergoing consistent training have adapted to their workload and do not have elevated protein needs. This effect may not have been evident in previous studies due to the use of untrained subjects, fasted conditions necessitated by metabolic tracers, and/or uncertainty associated with the assumptions made when using previous methods. Because previous studies have shown that untrained subjects do require additional protein to support LBM accretion during the adaptation response to exercise, protein requirements for these subjects may not be easily extrapolated to individuals undergoing chronic training. Studies that measure muscle metabolic processes in free-living, steady state, trained subjects do not exist; however, this type of study has the potential to greatly impact the field of sports nutrition.

In conclusion, the costs and benefits of high protein intakes need to be considered in light of the current standard of consuming large amounts of protein to reduce risk of deficiency rather than adhering to currently accepted optimal intakes. Lemon (61) cited some benefits of increased protein intakes, such as surplus amino acid intake during digestion of a high-protein diet aiding protein synthesis and/or minimizing protein breakdown, as well as greater nitrogen retention in novice bodybuilders consuming a high-protein diet (334% versus 124% of RDA). However, high protein intakes can also have drawbacks that can be detrimental to athletes and their

performance. These drawbacks include increased work by the kidneys, dehydration, storage of excess protein as fat, a high fat content potentially coupled with the protein, and increased calcium loss, which could accelerate osteoporosis, particularly in women (60). Optimal dietary protein intake should not cause excessive urea production or oxidative losses of amino acids beyond what is necessary for peak functioning (86). Because the amino acid pool size cannot be expanded beyond a certain point and nitrogen cannot be stored, intake above the threshold will simply be oxidized and/or excreted; both of these processes require energy above efficient expenditure. As such, large intakes of amino acids can interfere with absorption, contribute to metabolic imbalances, and alter brain neurotransmitter activity (61). When protein intake is excessively high, degradative pathways may be upregulated to accommodate this state so that a subsequent period of lower protein intake may be insufficient to offset losses until the body is able to adapt to the new, lower intake (75, 93). Habitual consumption of a high-protein diet may cause the increased need for dietary protein due to the adaptation of pathways for oxidative amino acid catabolism (74, 76, 93). Such accommodative processes may prevent adaptation from occurring by keeping the efficiency of amino acid recycling and uptake low. The efficient and optimal utilization of protein and amino acids will be key for elite athletes seeking to reach and maintain peak condition and thus perform at their highest level.

Future Issues

Based on the review of the literature in the field of muscle protein synthesis, the following points have been identified as areas of future interest:

1. Future studies should investigate the interaction effect between timing of and total protein intake to determine whether optimally timing intake in conjunction with the exercise-induced anabolic window affects total daily protein needs.
2. Future studies should determine the effect of training status on longer-term MPS response to exercise.
3. Future studies should determine the relationship between acute and chronic MPS responses to exercise.
4. Future studies should measure C-MPS and subsequent LBM accretion over longer periods in order to better understand the overall intervention effect.

Summary Points

The following points summarize the findings from a review of the literature in the area of muscle protein synthesis, total daily protein requirements, and timing of protein supplementation in conjunction with exercise:

1. Current protein intake recommendations for endurance athletes and resistance-training athletes are 1.2 to 1.4 g/kg per day and 1.6 to 1.8 g/kg per day, respectively.
2. Inconsistent results in timing and total protein effects on muscle metabolism and LBM accretion may be the result of differences in subject training status, fasted status of subjects, and questionable assumptions necessitated by previous methodologies.

3. Individuals in a stable training program have likely adapted to their workload and may not have elevated protein needs.
4. Although the effect of timing of protein intake in conjunction with exercise may be an artifact of the methodologies previously used to measure muscle protein synthesis acutely, if there is indeed an optimal window for nutrient ingestion, it is reasonable to hypothesize that supplementation at that time would result in a decreased total daily protein requirement.
5. It is unclear whether acute MPS responses can be extrapolated to LBM accretion following chronic RET.
6. Although radioactive metabolic tracer methodologies are currently the gold standard in measuring MPS, assumptions about precursor pool labeling and tracer behavior may not hold with exercise.
7. Because deuterium methods are not subject to the same assumptions and limitations as other metabolic tracers, deuterium may allow recommendations to be made that have better application to real-world scenarios.
8. Active individuals and athletes often follow an energy-restricted diet; therefore, optimal nutrient intake and efficiency of nutrient utilization are critically important to their ability to maximize their performance in both training and competition.

CHAPTER III

DESIGN AND METHODS

Subjects

Subjects (n=67) were recruited for this study from a pool of trained, healthy males between 18 and 29 years old. Subjects were recruited from Texas A&M University, and all subjects were screened prior to participation to ensure that they met study requirements for health and "trained" status. Subjects were required to have at least one year of involvement in resistance *and* aerobic training, which was evaluated by a validated physical activity questionnaire to assess their overall training status (**Appendix A6**). Subjects with cardiac abnormalities, history of blood thinning medication, chronic illnesses, and other pertinent health conditions were excluded (**Appendix A3**). First semester students, subjects less than 18 years old, females, and individuals with less than one year of resistance or aerobic training were excluded as well. Risks and benefits were explained to subjects (**Appendix A5**), who provided written consent (**Appendix A1 and A2**) to participate in this study in accordance with the Texas A&M Institutional Review Board (IRB2016-0376; IBC: 2016-018, 2014-054). Of the 67 volunteers, 46 (21.8±3.1 yr, 182.2±6.2 cm, 83.5±13.6 kg) completed the full requirements of the study. Subjects were allowed a maximum of two absences from the training protocol, and missed sessions were made up within the same week. Demographics for subjects completing the study in its entirety are shown in **Table 2**.

Design

A randomized double blind experimental design was employed. Participants were randomly assigned to low (**LO**) or high (**HI**) protein intake groups (1 $g^{-1}\cdot kg^{-1}\cdot day^{-1}$ and 2 $g^{-1}\cdot kg^{-1}\cdot day^{-1}$, respectively). These groups were further randomized to either supplementation immediately post-exercise (**IPE**), or supplementation three hours delayed post-exercise (**DPE**) (**Table 1**). Thus, there were four intervention groups: (1) Low Protein Delayed Supplementation (**LO/DPE**, n=9), (2) Low Protein Immediate Supplementation (**LO/IPE**, n=9), (3) High Protein Delayed Supplementation (**HI/DPE**, n=9), and (4) High Protein Immediate Supplementation (**HI/IPE**, n=9). Additionally, a non-intervention control group (**CON**) ingested protein according to their normal diet throughout the day to establish reference MPS measures (n=10).

	TIMING PROTEIN INTAKE	
TOTAL PROTEIN INTAKE	**DPE**	**IPE**
1 $g^{-1}\cdot kg^{-1}\cdot day^{-1}$	LO/DPE (n=9)	LO/IPE (n=9)
2 $g^{-1}\cdot kg^{-1}\cdot day^{-1}$	HI/DPE (n=9)	HI/IPE (n=9)

Table 1 - Experimental Design. High and low protein intake groups were supplemented either immediately post-exercise or three hours post-exercise.

This study lasted a total of four weeks (31 days). After recruitment and consent, subjects in the intervention groups began a two-week familiarization period to adjust to the prescribed exercise and nutrition protocols. Subjects then underwent baseline testing

before beginning the two-week intervention period. While subjects were exempt from any external physical training, they logged any and all physical activity on training days utilizing a 24-hour recall method (**Appendix A11**). Subjects also logged acute sleep patterns on training days as well as chronic sleep patterns at baseline and follow-up testing using validated Stanford (48a) and Pittsburgh (19a) questionnaires, respectively (**Appendix A12 and A13**). Muscle biopsies (**MB**) and deuterium (**D2O**) administration occurred during the final experimental days to measure muscle protein synthesis over the entire 24-hour post-exercise recovery period following the final exercise training session. Follow-up measurements were taken 48 hours after the final exercise bout (**Figures 7 and 8**).

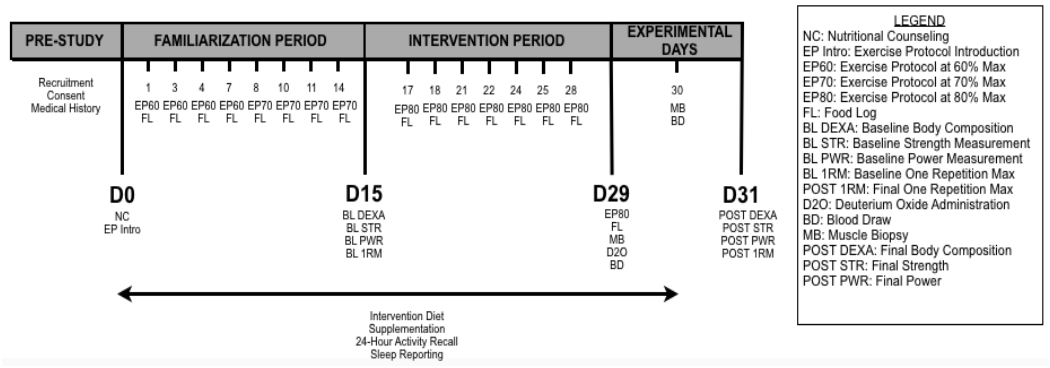

Figure 7 - Timeline Overview of Study Protocols. Subjects completed two weeks of familiarization with the exercise protocol before beginning two weeks of the intervention period. The experimental days began before the final exercise bout. Performance measures were taken before and after the intervention period.

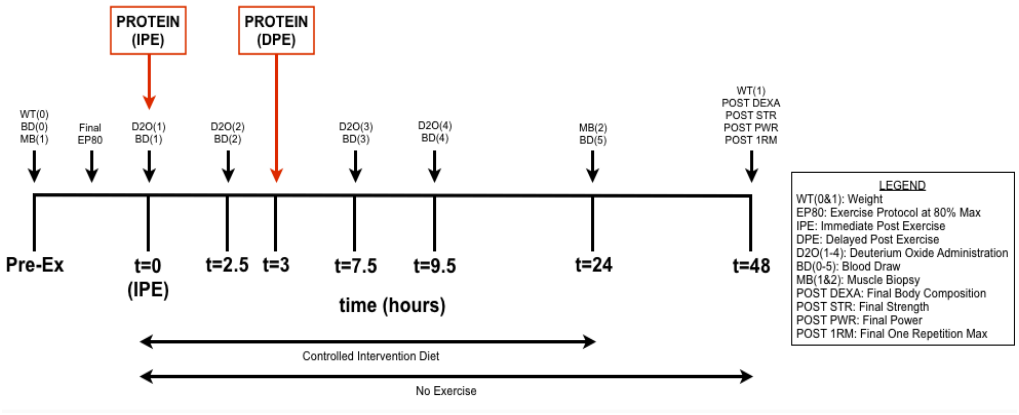

Figure 8 - Timeline of Experimental Days. Subjects received a muscle biopsy prior to the final bout of exercise, after which deuterium was consumed at intervals throughout the day. A second muscle biopsy was taken 24 hours post-exercise. Blood draws were performed in conjunction with all muscle biopsies and deuterium administration.

Exercise Protocol

Subjects completed an exercise protocol similar to that of Parr et al. (2014), which is a concurrent training model designed to simulate the key characteristics of sport training by including both resistance training and sprint interval training. Since the majority of sport training consists of intervals of intense interval activity combined with a strength-training regimen, this exercise protocol was designed to more closely reflect the activities of elite athletes in a typical training environment compared to aerobic or resistance exercise training protocols alone. Additionally, muscle protein synthesis has been shown to increase with sprint intervals performed on a cycle ergometer (83aa). Sprint intervals and resistance training combine to create a strength and power exercise protocol, which is the training state that has been recommended in the literature to

require the highest intake of dietary protein. This protocol was thus designed to simulate elite athlete training, maximize muscle protein synthesis, and provide the greatest potential to detect effects of total daily and timing of protein intake in a strength and power athlete.

On training days, subjects were asked to jog to the laboratory as part of their warm-up. They then conducted a series of dynamic stretches and plyometrics for three minutes. The sprint intervals were conducted on a cycle ergometer (Schwinn Airdyne, Nautilus, Inc., Vancouver, WA) and consisted of a two-minute warm-up followed by 10 sets of 30-second sprint intervals with 30 seconds of active recovery between each sprint bout. Sprint bouts were performed at 100% effort, measured as a rating of perceived exertion (**RPE**) of 10 using the OMNIResistance Exercise Scale (**Appendix A14**; 98a). The warm-up as well as active recovery were completed at 50% effort, or 5 RPEs. A two-minute cool-down at 50% effort was completed following the final sprint bout, and an additional 5 minutes of rest were given before beginning the resistance exercise component of the training session. The resistance-training component consisted of bench press, leg press, leg extension, and lat pulls (Keiser, Fresno, CA). Subjects performed a warm-up of five repetitions at 50% of the subject's one-repetition maximum (**1RM**), and five repetitions at 60% of the 1RM on each machine. Once warm-up was completed, subjects then completed three sets to failure at 80% of their 1RM with three minutes of recovery between each set. If at any point subjects performed less than 4 or more than 12 repetitions in one set, their prescribed weight was adjusted by 2% of their

1RM up or down, respectively, in order to maintain the prescribed intensity. This protocol was completed four times per week for two weeks (8 total sessions).

There was a two-week familiarization period to allow subjects to be introduced to the exercise protocol and equipment. The first four sessions of the familiarization period scaled the exercise protocol to approximately 60% of their maximal RPE, while the final four sessions were scaled to 70% maximal RPE. Baseline testing to determine 1RM was performed after the final familiarization session. The experimental diet was followed during this familiarization period to allow any adaptive processes to occur before the start of the intervention.

Nutrition

Food intake was controlled and monitored for these subjects by study personnel. Prior to the familiarization period, an online nutritional brief was viewed by subjects in order to educate their meal choices and provide instruction for logging meals. Researchers monitored food log entries on training days to verify appropriate serving sizes and macronutrient content. Supplementation for the intervention groups was provided in the form of whey protein (Vanilla Cor-Performance Whey, CelluCor, Bryan, TX) in the amount of 0.4 g protein per 1 kg of lean mass per dose. One intervention group received their supplement immediately post-exercise (IPE), while the other group received their supplement three hours delayed post-exercise (DPE). The group not receiving a protein supplement at a given time (immediate or delayed) did not receive any other supplementation and did not ingest any other foods during the three-and-one-half hour post-exercise time frame. The control group consumed and logged meals

normally over the course of the day without intervention. All groups maintained individualized isocaloric diets so that total energy intake was controlled. All meals were photographed and recorded on each training day, including training during the familiarization period, and all food logs (n=16 per person) were analyzed with Nutribase Professional 11 (Cybersoft, Phoenix, AZ) to ensure that diets were in accordance with study procedures.

On the experimental day (IPE + 24 hours), subjects were required to follow a prescribed diet with administered protein to precisely match their protein level and caloric intake. The non-intervention control group also followed a 2 g/kg/d diet on the experimental day to ensure maximal rates of muscle protein synthesis. Intake during the experimental day was verified by written food logs and photographed meals.

Performance Measures

Baseline measurements of body composition, strength, 1RM, and power were obtained following the familiarization period and prior to the start of the intervention period. Body composition, including whole body percent fat, fat mass, and lean body mass, were assessed using dual-energy X-ray absorptiometry (**DEXA**). Total thigh and thigh cross section composition were also determined from DEXA data as described by Hansen et al. (2007). Assessment of isometric and isokinetic strength for quadriceps and hamstring was conducted by measuring force production using an isokinetic dynamometer (Biodex Medical Systems, Shirley, NY) as validated by Drouin et al. (2004) and summarized by Harbo et al. (2012). Subjects obtained their 1RM on bench press and three repetition maximum (**3RM**) on the remaining resistance exercise

machines (leg press, lat pull, knee extension) as an additional measure of strength. For exercises where a 3RM was obtained, the 1RM was calculated using the Epley Equation (38a). Maximum power and relative power were also measured using a power bike (Power Cycle SS, Austin, TX) as described by Martin et al. (1997). Isometric and isokinetic strength and power measurements were all normalized to thigh lean mass (in kg) for analysis. All of these measurements were taken again one day after the 24-hour post-exercise muscle biopsy (48 hours after the final training session).

Control Group

A control group was established to provide a metabolic baseline for the intervention groups. Control subjects did not report to the lab for organized training but continued to exercise on their own as normal. Controls were active in the study for 15 days, beginning with baseline testing and ending with experimental days and follow-up testing. For each day the intervention group trained, controls logged activity to verify they were exercising as normal and completed food logs (8 days total). Controls received the muscle biopsies at the same time points as subjects in the intervention groups but did not perform exercise during this time. The control group followed the same protocols on the experimental days for blood draws, biopsies, deuterium administration, and standardized protein intake. Thus, the control group acted as a reference point for activity levels, diet, and normal rates of protein synthesis without exercise.

Blood Samples

Blood samples were taken in conjunction with deuterium administration and muscle biopsies. Blood samples were drawn without stasis from an antecubital vein with the subject seated at quiet rest into Vacutainer tubes containing 10.5 mg Na-EDTA for plasma collection. Plasma samples were immediately isolated by centrifugation at 2500 x g for 25 minutes at 4°C and stored at -80°C for later analysis. The first blood sample was taken prior to the first muscle biopsy and the start of the final bout of exercise to establish each subject's resting baseline. Plasma levels of deuterium were measured via blood draw immediately after each bolus of deuterium oxide to verify maintenance of appropriate levels. The final blood draw (**BD**) was taken 24 hours post-exercise immediately before the second muscle biopsy.

Muscle Biopsy

Muscle biopsies were obtained twice: once immediately before the final bout of exercise, and again 24 hours after the final bout of exercise. Biopsies were taken from the vastus lateralis under local anesthesia (1% Xylocaine HCl) using a 5-mm needle. All muscle samples were cleaned of visible fat, connective tissue, and blood. Muscle samples were immediately frozen in liquid nitrogen (−190ºC), and then stored at −80ºC until analyzed. Muscle biopsies were necessary to determine cumulative muscle protein synthesis based on the measurement of deuterium tracer incorporated into tissue. Subjects were given a list of medications to avoid that may increase bleeding risk in order to minimize complications with muscle biopsies (**Appendix A4**).

Muscle Protein Synthesis Using Deuterium

Deuterium, or "heavy water", can be used as a means of measuring muscle protein synthesis in free-living humans over longer time periods (58). D2O acts as a tracer by equilibrating within the body water pool in approximately 2 hours (44, 114). As hydrogen molecules are exchanged, deuterium is incorporated into multiple metabolic pools and tissues, including muscle. The slow rate of decay allows adequate concentrations to be maintained within body water over the course of at least one week, so that the deuterium can continue to be taken up by muscle to allow for accurate measurement of muscle protein synthesis. Myofibrillar fractional synthetic rate was determined from the incorporation of deuterium-labeled alanine into protein, which was measured using body water enrichment over the 24-hour recovery period following the final bout of exercise. Thus, cumulative protein synthesis was measured over the entire post-exercise period according to the following equation:

$$\text{FSR} = \left[\frac{^{2}\text{H} - \text{labeled Alanine in protein}}{(^{2}\text{H}_2\text{O plasma enrichment} * 3.7) * \text{time}_{\text{hours}}} \right] * 100$$

The following methods for administering and measuring deuterium have been previously published (42, 58). Subjects received a blood draw and their first of 4 boluses of 70% $^{2}H_2O$ (3 ml·kg bodyweight total) (Cambridge Isotopes, Andover, MA) to achieve approximately 0.4% to 0.8% ^{2}H-labeling of body water immediately following the final exercise bout. The remaining boluses were given 2, 7.5, and 9.5 hours

following exercise. Subjects returned to the laboratory 24h following cessation of exercise for another blood draw and vastus lateralis muscle biopsy.

Analysis of Myofibrillar FSR

Analysis of ^{2}H-labeling of body water was obtained from the blood draw, and protein-bound alanine was determined from the biopsy as previously described (42, 44, 44a, 58). In summary, 2.0 µL of 10N NaOH and 4.0 µL of a 5% solution of acetone in acetonitrile was added to 20 µL of plasma. After 24 hours, 600 µL of hexane was added to separate water from the sample and 200 µL were extracted for measurement. Muscle tissue was prepared by pulverizing the entire sample and measuring approximately 50 mg for subsequent homogenization. Samples were homogenized in 0.4 mL of 1x Norris Buffer with NaF and Na_3VO_4 and 1% Triton and allowed to settle on ice for one hour. Homogenates were then centrifuged at 14,000 rpm for 30 minutes at 4ºC. The supernatant containing the cytosolic portion was decanted and stored at -80ºC for future Western Blot analysis. The remaining muscle pellet was vortexed with 300 µL of TCA and homogenized once more. A second "soft" spin at 3800 rpm for 15 minutes at 4ºC was performed and the supernatant was decanted. The addition of TCA and subsequent soft spin were performed two additional times for a total of three rounds. After the final soft spin, 6N HCl was added in proportion to sample weight (6 µL per mg) and placed on a 100ºC heating block for 24 hours. After heating, 50 µL of the sample was dried down in the heating block for one hour before 50 µL of a 3-2-1 methyl-8/methanol/acetonitrile solution was added. The samples were incubated for one hour and transferred to GCMS vials. All samples were analyzed using an Agilent 5973-MSD

equipped with an Agilent 6890 GC system and a DB17-MS capillary column (30m x 0.25 mm x 0.25 μm).

Statistical Analysis

The statistical analysis of data collected from the study was broken down into preliminary and primary analyses.

Preliminary Analysis

Preliminary statistics were conducted to determine power and adequate sample size, identify statistical outliers, verify difference from controls, and validate the nutritional intervention.

Power

The number of subjects per group (n=9) was determined to meet the minimum sample size needed to detect differences in 24-hour fractional synthetic rates (%/day) between groups with a 1-β of 0.8, based on an estimated effect size of 0.68 standard deviation units (based on previous studies measuring FSR with deuterium) and a two-tailed alpha level of 0.05.

Outliers

Outlier tests were conducted in SPSS for all data sets overall and group by group. One control subject was identified as both an overall and group outlier for multiple data sets and was thus removed from all further analyses, bringing the control group count to 9 subjects and overall count to 45 subjects total.

Control

A preliminary analysis used four independent samples t-tests to compare the primary outcome (FSR) of the non-intervention control group to each of the four intervention groups, as well as an independent samples t-test to compare the combined training groups together to the control group. This analysis determined whether there was an overall exercise effect as well as effects of each intervention on FSR. Additionally, demographic data, MAQ, and sleep variables were analyzed with SPSS (IBM Corp. 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY) using a one-way ANOVA to ensure the study was well-controlled and groups were not significantly different in these areas prior to beginning the study.

Intervention Validation

Nutribase food logs were analyzed with SPSS (IBM Corp. 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY) using a one-way ANOVA to compare protein intake among groups and thus ensure that subjects followed their respective intervention diets. Other macro- and micro- nutrients were compared, as well as total calories, as these have the potential to affect muscle metabolic processes in response to exercise.

Primary Analysis

Primary analyses focused on the primary outcome of myofibrillar fractional synthetic rate as a measure of muscle protein synthesis, and the secondary outcomes of strength, power, and body composition as measures of performance.

Primary Outcomes

Fractional synthetic rates (specifically, myoFSR) for intervention groups were normalized a priori to non-exercising (over 24-hour period) controls to measure the relative increase in FSR above baseline. Normalized FSR was then analyzed using a 2 (total protein intake) x 2 (timing of post-exercise supplementation) independent samples ANOVA using SPSS (IBM Corp. 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY). When appropriate, a corrected Tukey's HSD post-hoc analysis was employed to identify differences between factors.

Secondary Outcomes

Performance and descriptive data, including 1RM, strength, power, and body composition, were analyzed using a one-way ANOVA, with application of a corrected Tukey HSD post-hoc analysis when appropriate.

CHAPTER IV

RESULTS

Demographics

Baseline characteristics of subjects in each group are shown in **Table 2**; no significant differences among groups were found (**Appendix B1**). Analysis of the Modified Activity Questionnaire (MAQ) showed that subjects were active 10.4±5.6 hours per week in the past year and had an average lifetime activity level of 18.8±13.8 hrs/wk (**Table 3**). Of that activity, strength training made up 4.2±3.0 hrs/wk in the past year and 3.7±4.4 hrs/wk overall (**Table 4**). Statistical analysis of the MAQ found no significant differences among groups in overall activity or strength training history (**Appendix B2 and B3**, respectively). There were also no significant differences between acute sleepiness among training groups or between baseline and follow-up measures of chronic sleep patterns (**Appendix B4**). The average Stanford Sleepiness Scale (**SSS**) was a 2.4±0.8, and the Pittsburgh Sleep Quality Index (**PSQI**) was 5.0±2.1 and 4.3±2.0 at baseline and follow-up, respectively (**Table 5**). All subjects were determined to be weight stable, with an average visit-to-visit weight variation of 0.8% (range: 0.3-1.5%).

	Age		Height		Weight	
Group	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	22.0	3.3	185.1	6.5	92.4	16.9
2 (LO/IPE)	22.1	3.4	182.3	7.9	81.5	11.9
3 (HI/DPE)	20.5	3.3	181.5	6.5	75.5	9.0
4 (HI/IPE)	22.9	3.6	180.9	5.8	81.1	7.3
5 (CON)	21.5	2.3	181.3	4.6	85.1	17.6
Total	21.8	3.2	182.2	6.2	83.2	13.9

Table 2 – Demographic Characteristics of Five Groups. Means of age (years), height (cm), and weight (kg) are given for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). Overall means for the entire study population (Total) are also shown. There were no significant differences among groups for any measure (Appendix B1).

	Past Year				Age 12-18				Age 19-34				Lifetime			
	hr/wk		Met-hr		hr/wk		Met-hr		hr/wk		Met-hr		hr/wk		Met-hr	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	8.6	3.9	45.2	18.2	13.5	12.2	80.5	70.6	3.4	3.4	18.0	18.1	14.6	15.1	85.5	86.7
2 (LO/IPE)	7.9	5.2	38.3	24.2	14.0	7.5	91.2	39.7	12.2	9.7	71.7	60.1	24.9	16.6	154.9	93.2
3 (HI/DPE)	10.9	6.4	85.9	115.8	13.5	8.1	91.4	60.8	7.1	8.1	51.1	77.2	19.7	12.7	136.1	121.1
4 (HI/IPE)	11.6	3.9	53.6	15.8	8.5	5.7	48.1	40.8	7.2	7.4	32.6	30.6	15.7	10.4	80.8	51.6
5 (CON)	13.3	7.2	71.4	51.9	9.7	7.3	73.9	93.6	12.0	8.5	62.7	43.5	18.7	13.7	120.9	111.5
Total	10.4	5.6	58.7	58.5	11.9	8.3	77.4	62.6	8.4	8.0	47.1	51.5	18.8	13.8	115.9	95.4

Table 3 – Overall Modified Activity Questionnaire. Results of the MAQ for the last year, ages 12-18, ages 19-34, and lifetime are given in hours per week (hr/wk) and Met-hours (Met-hr) for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). Overall means for the entire study population (Total) are also shown. There were no significant differences among groups for any measure at any time point (Appendix B2).

	Past Year				Age 12-18				Age 19-34				Lifetime			
	hr/wk		Met-hr		hr/wk		Met-hr		hr/wk		Met-hr		hr/wk		Met-hr	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	2.5	2.2	11.3	10.0	0.8	1.7	3.7	7.6	0.5	1.0	2.3	4.5	1.1	2.4	4.9	10.6
2 (LO/IPE)	3.5	2.1	15.5	9.7	1.1	1.0	4.9	4.3	2.4	1.7	10.9	7.4	3.4	2.1	15.3	9.3
3 (HI/DPE)	4.7	3.7	21.0	16.7	2.7	3.2	12.2	14.5	2.0	3.2	8.9	14.4	4.7	5.7	21.1	25.8
4 (HI/IPE)	5.3	2.4	23.8	10.6	0.9	1.0	4.1	4.4	2.7	2.5	11.9	11.2	3.6	2.8	16.1	12.6
5 (CON)	5.1	3.7	23.0	16.6	2.3	2.2	10.2	10.0	3.9	4.5	17.7	20.3	6.2	6.5	27.8	29.2
Total	4.2	3.0	18.9	13.3	1.6	2.1	7.1	9.2	2.3	2.9	10.4	13.0	3.7	4.4	16.7	19.6

Table 4 – Strength Training Modified Activity Questionnaire. Results of the MAQ for strength training for the last year, ages 12-18, ages 19-34, and lifetime are given in hours per week (hr/wk) and Met-hours (Met-hr) for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). Overall means for the entire study population (Total) are also shown. There were no significant differences among groups for any measure at any time point (Appendix B3).

	Stanford Sleepiness Scale		Pittsburgh Sleep Quality Index			
			Baseline		*Follow-Up*	
Group	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	2.7	1.4	4.2	2.2	3.4	2.2
2 (LO/IPE)	2.5	0.7	5.4	2.8	4.8	2.3
3 (HI/DPE)	2.5	0.3	5.8	1.6	4.9	2.1
4 (HI/IPE)	2.1	0.6	5.0	1.3	4.4	1.4
5 (CON)	-	-	4.8	2.5	4.2	2.0
Total	2.4	0.8	5.0	2.1	4.3	2.0

Table 5 – Acute and Chronic Sleep Pattern Measures. Mean acute sleepiness, represented by the Stanford Sleepiness Scale, and mean chronic sleep quality, represented by the Pittsburgh Sleep Quality Index, are given in scale units for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). Overall means for the entire study population (Total) are also shown. There were no significant differences among groups for any measure at any time point (Appendix B4).

Nutrition

Food logs for subjects engaged in training were analyzed separately for the familiarization period (**FAM**) and the intervention period (**INT**) (**Table 6**). There were no significant differences between the familiarization and intervention diet within the low and high protein groups (**Appendix B5 and B6**, respectively). The low and high protein intake intervention groups were compared to controls, as seen in **Table 7**.

Low protein groups consumed a significantly lower average calorie intake of 2031±593 versus 2655±489 calories in the high protein group ($p=0.013$, **Appendix B7 and B8**). Low protein groups consumed a diet of 24±5, 43±9, 33±7% of calories from protein/carbohydrate/fat compared to 27±5, 42±5, 31±5% in high protein groups, and 22±4, 43±8, 33±6% in controls.

Protein consumption relative to total body weight was an average of 1.3±0.3 g/kg/day in the low protein groups compared to 2.2±0.3 g/kg/day in the high protein groups ($p<0.001$, 95% CI [-1.2, -0.5]), while control group protein intake fell between the two intervention groups at 1.6 g/kg/day, which was significantly lower than the high protein group ($p=0.002$, 95% CI [-1, -0.2]).

Protein consumption relative to lean mass was significantly lower for the low protein groups at 1.9±0.5 g/kg/day compared to the high protein group that ingested 2.7±0.5 g/kg/day ($p=0.002$, 95% CI [-1.2, -0.3]. Again, the control group fell between the high and low protein groups and consumed 2.1±0.9 grams of protein per kg lean mass per day but was not significantly different from either intervention group.

A Tukey HSD post-hoc test revealed that the low protein group ingested significantly less Omega-6 (p=0.046), ash (p=0.006), choline (p=0.01), Vitamin D3 (p=0.014), gamma tocopherol (p=0.012), magnesium (p=0.021), potassium (p=0.031), and selenium (p=0.001) compared to controls (**Appendix B8**). Low protein groups ingested significantly less cholesterol than high protein groups (p=0.043) and control group (p=0.001). Retinol, Vitamin D, Vitamin D2+D3, delta tocopherol, Vitamin K1D, and phosphate were all lower in intervention groups than controls but were not significantly different from each other (**Appendix B8**). Little amino acid data was available for analysis in Nutribase, but differences in logged amino acids can be found in **Appendix B6a**. Furthermore, comprehensive nutritional analyses of all assessed nutrients for each of the four intervention groups and control group are shown in **Appendix B9-11**.

		Calories		Energy(kj)		Protein(g)		Protein(g/kg TOTAL)		Protein(g/kg LEAN)	
Group	Period	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	FAM	2233	656	9342	2744	122	30	1.4	0.4	1.9	0.5
	INT	2031	593	8490	2486	112	21	1.3	0.3	1.8	0.3
High Protein	FAM	2557	635	10696	2655	162	37	2.1	0.4	2.7	0.5
	INT	2655	489	11106	2047	170	31	2.2	0.3	2.8	0.5

		Carbohydrate(g)		Fat(g)		%Calories/Protein		%Calories/Carbohydrate		%Calories/Fat	
Group	Period	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	FAM	266	89	79	31	23	6	44	9	32	7
	INT	239	84	74	27	24	5	43	9	33	7
High Protein	FAM	273	81	93	29	26	4	40	7	33	4
	INT	299	73	89	22	27	5	42	5	31	5

Table 6 – Summary of Nutrition for Low and High Protein Groups During Familiarization and Intervention Periods. Calorie and macronutrient intake during familiarization and intervention periods compared for combined low and combined high protein groups. There were no significant differences among groups for any measure at any time point (Appendix B5 and B6).

Calorie Information		Calories		Energy(kj)		Calories/Protein		%Calories/Protein		Calories/Carbohydrate		%Calories/Carbohydrate		Calories/Fat		%Calories/Fat	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	2031[a]	593	8490[a]	2486	459[a]	90	24[ab]	5	882	326	43	9	684	259	33	7
High Protein	18	2655[b]	489	11106[b]	2047	696[b]	127	27[a]	5	1122	274	42	5	826	208	31	5
Control	9	2429[ab]	908	10165[ab]	3798	534[a]	210	22[b]	4	1017	318	44	9	878	482	35	8
Total	45	2360	677	9871	2838	569	171	25	5	1005	317	43	8	780	302	33	6

Macronutrients		Protein(g)		Protein(g/kg TOTAL)		Protein(g/kg LEAN)		Carbohydrate(g)		Fat(g)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	112[a]	21	1.3[a]	0.3	1.9[a]	0.5	239	84	74	27
High Protein	18	170[b]	31	2.2[b]	0.3	2.7[b]	0.5	299	73	89	22
Control	9	130[a]	51	1.6[a]	0.7	2.1[ab]	0.9	268	83	95	53
Total	45	139	42	1.7	0.6	2.2	0.7	268	83	84	33

Micronutrients		Cholesterol(mg)		Ash(g)		Theobromine(mg)		Choline(mg)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	195	68	2.7	2.2	2	5	30	25
High Protein	18	384	180	4.9	3.7	3	9	122	130
Control	9	564	437	8.2	7.1	25	48	278	406
Total	45	345*	262	4.7*	4.5	7*	23	116*	213

Vitamins		Retinol(mcg)		Vit-D(IU)		Vit-D3(mcg)		Vit-D2+D3(mcg)		GammaTocopherol(mg)		DeltaTocopherol(mg)		Vit-K1D(mcg)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	53[a]	64	35[a]	61	0.1[a]	0.3	0.2[a]	0.5	0.6[a]	0.8	0[a]	0	1[a]	2.9
High Protein	18	61[b]	81	66[a]	65	0.7[ab]	1.2	0.8[a]	1.2	1.4[ab]	1.9	0.1[a]	0.3	1.1[a]	2.0
Control	9	190[b]	202	163[b]	169	2.3[b]	3.8	2.9[b]	3.8	2.4[b]	1.9	0.4[b]	0.5	4.1[b]	4.7
Total	45	84	120	73	103	0.8	2.0	1.0	2.1	1.3	1.7	0.1	0.3	1.6	3.2

Minerals		Magnesium(mg)		Phosphate(mg)		Potassium(mg)		Sodium(mg)		Selenium(mcg)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	66[a]	59	226[a]	154	711[a]	492	2901	1014	12[a]	10
High Protein	18	118[ab]	85	456[a]	314	1067[ab]	653	3874	1243	43[ab]	40
Control	9	165[b]	133	876[b]	754	1475[b]	1113	4013	1822	74[b]	69
Total	45	107	94	448	456	1006	752	3512*	1362	37	45

Table 7 – Summary of Nutrition for Three Groups. Macronutrients and significant micronutrients compared for three groups: Low Protein, High Protein, and Control. Overall means for the entire study population (Total) are also shown. Significant differences among groups were found for total calories, energy, total calories from protein, %calories from protein, total grams of protein, grams per kg total body mass per day protein, grams per kg lean mass per day protein, and all displayed micronutrients (* = ANOVA $p<0.05$; [ab] = Tukey $p<0.05$). See Appendix B6a for full table, and Appendix B7 and B8 for analyses.

Performance Measures

Analysis of thigh cross section from DEXA data was conducted by two researchers with an intertester correlation of 99%. Total body percent fat and lean mass were not significantly different at baseline or follow-up among groups, nor was there a significant change between the two time points for either measure (**Table 8**, **Appendix B12**). However, change in thigh percent fat ($p=0.002$) and percent change in total thigh fat mass ($p=0.034$) were significantly greater in the LO/DPE group compared to controls (**Tables 9-10**, **Appendix B13-15**).

ANOVA found significant differences among groups in change in cross section percent fat ($p=0.002$) and percent change in thigh cross section fat mass ($p=0.033$) but Tukey HSD did not detect group differences. Baseline thigh fat mass was higher in the LO/DPE group compared to HI/DPE (2820.6±1325.6 vs. 1615.5±629.1 grams, $p=0.05$) and HI/IPE (2820.6±1325.6 vs. 1587.5±454.5 grams, $p=0.047$), and baseline cross section fat mass was higher in the LO/DPE group compared to HI/DPE (74.1±38.7 vs. 38.2±16.5 grams, $p=0.045$; **Appendix B15**). Total thigh lean mass and thigh cross section lean mass were not significantly different among groups.

Normalized isokinetic knee extension strength was significantly greater at baseline in the LO/IPE and HI/DPE groups compared to controls ($p=0.044$ and $p=0.024$, respectively; **Table 11**, **Appendix B16 and B18**). A paired samples test found that normalized power increased from baseline to follow-up for all groups ($p<0.001$), but there were no significant differences among groups at baseline or follow-up for normalized isokinetic knee flexion strength, isometric strength, or power.

Analysis of 1RM (in total pounds) found absolute knee extension at follow-up was significantly greater in the LO/DPE group compared to controls (250.4±25.4 vs. 192.2±31.4, p=0.006), and the percent change in knee extension 1RM was greater in the HI/DPE group compared to controls (p=0.006) (**Appendix B17-18**).

An independent samples test found significantly greater percent increase in leg press 1RM and knee extension 1RM in the combined intervention groups vs. controls (p=0.027 and p=0.006, respectively), but no other significant differences were found among groups for 1RM exercise testing at baseline or follow-up.

	Total Body Composition											
	% Fat						Lean Mass (kg)					
	Baseline		*Follow-Up*		*Change*		*Baseline*		*Follow-Up*		*Change*	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	24.2	5.9	23.6	5.7	-0.6	0.6	66.5	8.7	67.3	8.7	0.7	0.9
2 (LO/IPE)	20.0	5.8	19.8	6.0	-0.2	1.1	62.6	9.7	62.4	9.1	-0.2	1.0
3 (HI/DPE)	18.8	6.3	18.9	5.9	0.1	0.8	58.6	7.2	59.1	7.4	0.5	0.6
4 (HI/IPE)	18.7	3.4	18.6	3.1	-0.1	0.7	63.2	4.9	63.1	4.3	-0.1	1.3
5 (CON)	18.7	3.4	21.6	7.1	0.2	1.1	63.0	10.6	63.1	10.4	0.1	1.6
Total	21.4	6.7	20.5	5.8	-0.1	0.9	62.8	8.5	63.0	8.3	0.2	1.1

Table 8 – Total Body Composition for Five Groups. Values at baseline and follow-up and change between the two time points are given for total body percent fat and lean mass for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). Overall means for the entire study population (Total) are also shown. There were no significant differences among groups for any measure at any time point (Appendix B12).

	Total Thigh Composition																	
	Thigh % Fat						Thigh Fat Mass (grams)						Thigh Lean Mass (grams)					
	Baseline		*Follow-Up*		*Change*		*Baseline*		*Follow-Up*		*%Change*		*Baseline*		*Follow-Up*		*%Change*	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	23.2	6.6	22.1	6.6	-1.1^{a}	1.2	2820.6^{a}	1325.6	2690.5	1285.5	-4.5^{a}	5.7	8796.0	1328.6	8919.1	1291.9	1.6	3.6
2 (LO/IPE)	18.3	4.9	18.0	5.4	-0.3^{ab}	1.2	1786.8^{ab}	549.8	1783.8	615.9	-1.0^{ab}	8.6	7971.4	1331.1	8089.7	1386.1	1.5	1.7
3 (HI/DPE)	17.4	5.7	17.3	5.5	-0.1^{ab}	0.9	1615.5^{b}	629.1	1606.6	614.7	0.0^{ab}	5.8	7539.8	1148.1	7565.8	1167.7	0.4	3.7
4 (HI/IPE)	16.3	3.5	15.8	3.2	-0.4^{ab}	0.9	1587.5^{b}	454.5	1563.3	449.0	-1.5^{ab}	4.1	8040.8	960.0	8156.3	932.6	1.5	3.0
5 (CON)	19.9	7.0	20.8	7.3	0.9^{b}	0.6	2194.3^{ab}	1201.6	2298.7	1283.3	4.6^{ab}	3.6	8180.4	1575.7	8113.8	1617.0	-0.9	1.9
Total	19.0	5.9	18.8	6.0	-0.2	1.1	2000.9	983.0	1988.6	984.9	-0.5	6.3	8105.7	1292.5	8168.9	1313.6	0.8	3.0

Table 9 – Total Thigh Composition for Five Groups. Values at baseline and follow-up and percent change between the two time points are given for total thigh percent fat, total thigh fat mass, and total thigh lean mass for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). Overall means for the entire study population (Total) are also shown. Significant differences among groups were found for change in thigh percent fat, baseline thigh fat mass, and percent change in thigh fat mass (^{ab} = Tukey $p<0.05$, Appendix B13 and B15).

	Thigh Cross Section Composition																	
	Cross Section % Fat						Cross Section Fat Mass (grams)						Cross Section Lean Mass (grams)					
	Baseline		*Follow-Up*		*Change*		*Baseline*		*Follow-Up*		*%Change*		*Baseline*		*Follow-Up*		*%Change*	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	18.3	6.5	17.6	6.3	-0.6	1.6	74.1[a]	38.7	71.1	37.8	-3.8	9.8	311.1	43.9	311.9	40.0	0.5	3.8
2 (LO/IPE)	14.3	4.6	13.3	4.6	-1.0	2.0	47.1[ab]	18.6	44.7	19.2	-5.0	15.7	280.7	41.6	288.1	43.4	2.7	2.8
3 (HI/DPE)	12.2	4.5	13.1	5.1	0.9	1.1	38.2[b]	16.5	41.8	18.6	9.4	8.1	270.3	35.8	271.1	32.9	0.5	4.1
4 (HI/IPE)	11.9	2.6	11.4	3.4	-0.5	1.5	40.4[ab]	12.1	39.3	14.3	-4.2	13.4	294.3	34.3	298.3	31.0	1.5	2.6
5 (CON)	15.5	6.4	16.3	6.7	0.9	1.3	59.5[ab]	34.3	63.7	37.0	7.5	11.2	302.2	52.3	303.3	49.7	0.5	3.0
Total	14.4	5.4	14.4	5.6	-0.1*	1.7	51.9	28.4	52.1	29.0	0.8*	13.1	291.7	42.8	294.5	40.7	1.1	3.3

Table 10 – Thigh Cross Section Composition for Five Groups. Values at baseline and follow-up and change between the two time points are given for thigh cross section percent fat, thigh cross section fat mass, and thigh cross section lean mass for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). Overall means for the entire study population (Total) are also shown. Significant differences among groups were found for change in thigh cross section percent fat, baseline cross section fat mass, and percent change in cross section fat mass (* = ANOVA $p<0.05$; [ab] = Tukey $p<0.05$, Appendix B14 and B15).

	Normalized Isokinetic Flexion						Normalized Isokinetic Extension					
	Baseline		*Follow-Up*		*%Change*		*Baseline*		*Follow-Up*		*%Change*	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	10.0	2.0	10.1	1.6	2.5	12.5	19.9[ab]	3.0	20.0	3.3	0.7	10.0
2 (LO/IPE)	10.6	1.3	10.7	1.9	1.8	14.2	20.4[a]	2.2	21.0	3.4	2.7	8.8
3 (HI/DPE)	10.5	1.6	10.4	2.1	-0.5	17.9	21.0[a]	2.0	18.8	3.6	-10.3	17.4
4 (HI/IPE)	9.4	1.9	10.5	2.2	12.1	6.9	19.7[ab]	3.2	20.2	3.3	2.8	5.9
5 (CON)	8.2	1.5	8.9	2.1	7.4	13.3	16.3[b]	2.7	16.9	2.9	4.1	13.9
Total	9.8	1.8	10.2	2.0	4.5	13.4	19.6	2.9	19.6	3.4	0.1	11.9

	Normalized Isometric						Normalized Power (Right Leg)					
	Baseline		*Follow-Up*		*%Change*		*Baseline*		*Follow-Up*		*%Change*	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	23.3	3.4	22.7	5.3	-3.2	16.5	130.3	19.7	141.7	16.1	9.5	9.0
2 (LO/IPE)	22.4	3.4	21.4	2.9	-3.2	17.7	146.8	14.2	153.4	12.6	5.2	11.9
3 (HI/DPE)	21.2	3.1	20.4	4.9	-4.1	16.4	132.0	16.9	144.5	14.7	10.1	9.2
4 (HI/IPE)	23.1	3.9	22.1	4.5	-4.5	5.0	137.2	11.9	146.9	15.4	7.4	10.9
5 (CON)	20.2	3.6	19.2	3.9	-4.9	7.0	125.4	12.5	132.8	23.2	6.1	17.6
Total	22.2	3.5	21.3	4.3	-3.9	13.3	134.3	16.4	143.9	17.4	7.7	11.7

Table 11 – Normalized Strength and Power for Five Groups. Values at baseline and follow-up and percent change between the two time points are given for normalized isokinetic flexion and extension strength (ft-lbs/kg thigh lean mass), isometric strength (ft-lbs/kg thigh lean mass), and right leg power (Watts/kg thigh lean mass) for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). Overall means for the entire study population (Total) are also shown. Significant differences among groups were found for baseline normalize isokinetic knee extension ([ab] = Tukey $p<0.05$, Appendix B16 and B18).

	Bench Press 1RM						Leg Press 1 RM					
	Baseline		*Follow-Up*		*%Change*		*Baseline*		*Follow-Up*		*%Change*	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	223.3	39.2	238.3	38.6	7.1	4.7	1145.6	256.1	1338.3	415.5	15.1	10.2
2 (LO/IPE)	215.6	56.4	231.7	56.6	7.8	3.2	1087.5	306.8	1282.6	503.4	16.1	14.4
3 (HI/DPE)	199.4	45.9	212.8	52.4	6.5	4.0	976.5	131.9	1049.2	131.2	7.6	4.4
4 (HI/IPE)	222.2	54.7	238.9	50.6	8.4	4.9	981.9	117.6	1135.0	167.4	15.6	10.1
5 (CON)	199.4	51.9	210.0	57.7	5.0	2.8	1022.8	310.4	1054.5	276.0	4.3	12.3
Total	212.0	48.9	226.3	50.8	7.0	4.0	1042.9	237.8	1171.9	337.5	11.7	11.4

	Lat Pull Down 1RM						Knee Extension 1RM					
	Baseline		*Follow-Up*		*%Change*		*Baseline*		*Follow-Up*		*%Change*	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	211.7	42.4	231.1	45.8	9.4	4.0	217.8	14.5	250.4[a]	25.4	15.0	9.0
2 (LO/IPE)	218.4	55.1	244.1	62.1	11.9	5.1	196.8	36.9	229.9[ab]	39.9	17.2	6.0
3 (HI/DPE)	202.3	43.6	229.6	57.0	13.0	8.0	187.0	33.8	220.3[ab]	40.4	18.7	15.6
4 (HI/IPE)	214.9	28.3	237.1	27.5	10.7	7.1	202.4	26.2	231.7[ab]	29.3	14.7	6.7
5 (CON)	193.2	44.3	213.1	39.8	12.9	24.5	180.3	26.1	192.2[b]	31.4	6.4	5.7
Total	208.1	42.6	231.0	47.0	11.6	11.8	196.8	30.2	224.9	37.6	14.4	9.9

Table 12 – One Repetition Maximum Testing Results for Five Groups. Values at baseline and follow-up and percent change between the two time points are given for bench press, leg press, lat pull down, and knee extension 1RM (lbs) for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). Overall means for the entire study population (Total) are also shown. Significant differences among groups were found for knee extension 1RM at follow-up ([ab] = Tukey $p<0.05$, Appendix B17 and B18).

FSR

Myofibrillar fractional synthetic rates over the 24-hour measurement period are shown for the combined intervention groups and the control group in Table 13. Levene's Test of Equal Variances was rejected, therefore equal variances were not assumed for the independent samples t-test and FSR was significantly greater in the combined training groups compared to controls (p=0.009, 95% CI [3.4, 21.5], **Appendix B19**). Individual intervention groups were also compared to the control group (**Table 14**), but only the LO/DPE group exhibited a significantly greater FSR compared to the control group that did not exercise during the 24-hour protein synthesis measurement period (p=0.043, 95% CI [0.9, 43.6], **Appendix B20**).

		FSR For Combined Intervention vs. Control Groups		
Group	N	Mean	SD	SEM
INT	36	28.6[a]	19.7	3.3
CON	9	16.1[b]	8.9	3.0

Table 13 – FSR for Combined Intervention Groups vs. Control. Mean myofibrillar fractional synthetic rates (%/day) are given for the combined intervention groups (n=36) compared to control group (n=9). The combined intervention group FSR was significantly greater than control group FSR (* = Independent Samples t-test p<0.05, Appendix B19).

			FSR for Individual Intervention and Control Groups		
Group No.	Group Name	N	Mean	SD	SEM
1	LO/DPE	9	38.4[a]	27.2	9.1
2	LO/IPE	9	26.6	16.1	5.4
3	HI/DPE	9	26.7	17.1	5.7
4	HI/IPE	9	22.6	15.9	5.3
5	CON	9	16.1[b]	8.9	3.0

Table 14 – FSR for Individual Intervention and Control Groups. Mean myofibrillar fractional synthetic rates (%/day) are given for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). The LO/DPE group FSR was significantly greater than control group FSR (* = Independent Samples t-test $p<0.05$, Appendix B20).

Fractional Synthetic Rates of intervention groups normalized to controls are summarized in **Table 15** as well as in **Figure 9**. There were no significant main or interaction effects of total protein intake and timing of supplementation on FSR (**Appendix B21-22**). Pairwise comparisons of High and Low total protein intake as well as Immediate and Delayed supplementation did not yield significant results (Appendix B23-24), nor did univariate tests of Protein and Timing groups (**Appendix B25-26**).

FSR for Intervention Groups Normalized to Control				
Protein Level	Supplementation Time	Mean	SD	N
HI	DPE	1.7	1.1	9
	IPE	1.4	1.0	9
	Total	1.5	1.0	18
LO	DPE	2.4	1.7	9
	IPE	1.6	1.0	9
	Total	2.0	1.4	18
Total	DPE	2.0	1.4	18
	IPE	1.5	1.0	18
	Total	1.8	1.2	36

Table 15 – FSR for Intervention Groups Normalized to Control. Means of myofibrillar fractional synthetic rates for total protein (low vs. high) and timing of supplementation (delayed vs. immediate) normalized to control group means. Means shown represent fold difference from control group. There were no significant differences among groups (Appendix B21).

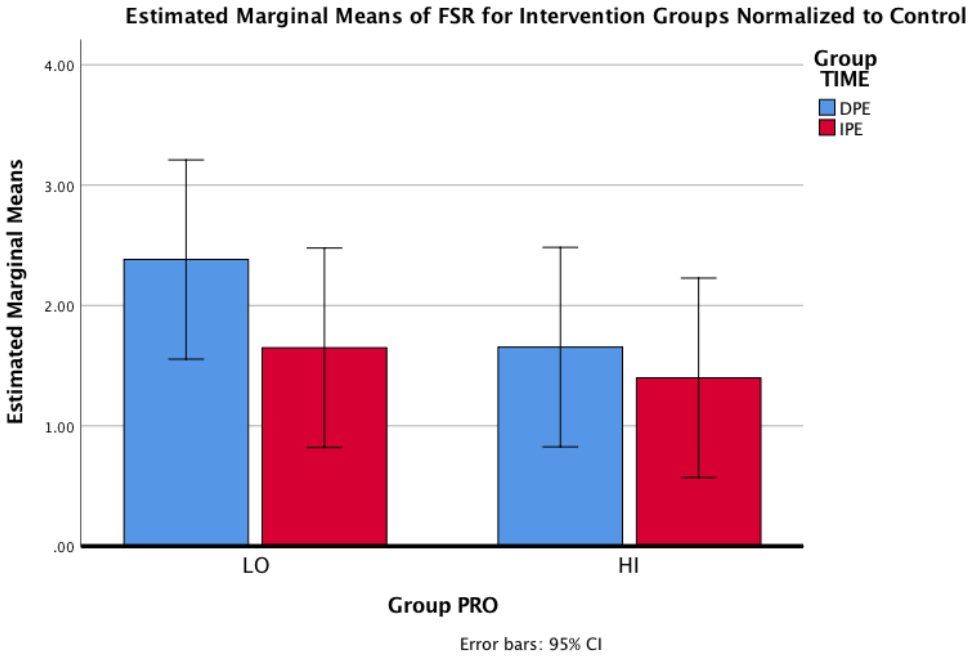

Figure 9 – Mean FSR for Intervention Groups Normalized to Control. Means of myofibrillar fractional synthetic rates for total protein (low vs. high) and timing of supplementation (delayed vs. immediate) normalized to control group means. There were no significant differences among groups. Means shown represent fold difference from control group. Delayed supplementation is shown in blue while immediate supplementation is shown in red. Error bars represent 95% confidence intervals.

CHAPTER V

CONCLUSION

Primary Outcomes

The novel finding of this study was that no significant differences in muscle protein synthesis were detected between high and low daily protein intake or timing of protein supplement ingestion following exercise in a study of 36 young well-trained males who engaged in 4 weeks of concurrent, simulated elite athlete training (control group n=9). This conclusion is contrary to the currently accepted dogma that greater protein intake is necessary for strength and power athletes, and that supplementation immediately post-exercise results in an elevated anabolic condition.

This study was well-controlled using an age- and activity-matched control group which served as a baseline to indicate the initial metabolic state of all other groups prior to dietary and supplementation interventions imposed by the study. This status of uniformity among groups at baseline was ensured by screening processes and verified by multiple measurements, including demographic data, activity levels, sleep patterns, body composition data, and strength and power measurements. No significant differences were found in demographic data including height, weight, and age. Likewise, acute and chronic sleep patterns were similar among groups. Analysis of the MAQ indicated no significant differences among groups for total activity levels or strength training history in the past year or over their lifetime, and all groups were highly active across a variety of sports and training modes. Total body percent fat and lean mass were not significantly different among groups, although baseline thigh fat mass was higher in the LO/DPE group compared to HI/IPE, and baseline cross section fat mass was higher in the

LO/DPE group compared to HI/DPE. Elevated fat mass in the thigh region prior to the start of the intervention period should not have affected primary outcomes of the study. Most importantly, lean mass in the thigh region was similar among groups. Normalized isokinetic knee extension strength was significantly greater at baseline in the LO/IPE and HI/DPE groups compared to controls, but all other measures of isokinetic or isometric strength, 1RM, and power were similar among groups at baseline. While the control group did not perform exercise on the experimental day, they were highly active and engaged in regular training on their own over the course of the two-week period between baseline and follow-up testing. Specifically, individuals in the control group were engaged in either military ROTC training (n=5), or university-affiliated club sports including weight lifting, rowing, and wrestling (n=4).

This study did not investigate training effects but focused on dietary and supplemental intervention. Subjects recruited to the study were already engaged in both cardiovascular and resistance training with a minimum training history of one year. The purpose of the training protocol was to create a uniform, high-intensity stimulus among all intervention groups prior to measurement of muscle protein synthesis. Standardized training allowed individuals to adapt to the exercise protocol during the two-week familiarization period and maintain a high level of exercise intensity throughout the two-week intervention period of the study. Previous research has indicated that adaptation to exercise occurs over 14-16 days (18); however, our study lasted for 31 days and included 16 training days, thus ensuring adequate time for both learning and adapting to the established training protocol. Concurrent training of sprint intervals followed by resistance exercise mimicked the format of workouts followed by elite athletes and developed subjects into well-conditioned strength and power athletes. Thus, the requirements and timeline

of the study ensured a steady-state, high-intensity training environment for fit, young males with verified training history.

The combined intervention groups were compared to the control group a priori with an independent samples t-test to establish that there was indeed an effect of exercise on mean FSR over the 24-hour measurement period. The combined intervention groups did in fact exhibit an FSR significantly higher than the non-exercising control group. However, when intervention groups were compared to the control group individually, only LO/DPE was significantly different from controls. While all exercised groups exhibited greater mean FSR compared to the unexercised control group, the mean difference was significantly greater for the LO/DPE intervention group vs. controls.

Intervention groups were normalized to control group mean FSR to provide a measure of the relative increase in FSR above the baseline represented by the age- and activity- matched control group that refrained from exercise on the experimental day. As seen in Figure 9, mean normalized FSR was higher for the LO/DPE group, although this difference did not achieve significance. Therefore, in conclusion, no differences in myofibrillar FSR were identified among groups that either consumed high or low protein or consumed the protein either immediately after exercise or delayed 3 hours.

Secondary Outcomes

Differences between low and high protein groups were verified at 1.3 and 2.2 g/kg/day (1.9 and 2.7 g/kg lean mass/day), respectively, with the control group protein intake falling at a moderate intake of 1.6 g/kg/day (2.1 g/kg lean mass/day). Food logs during the familiarization period were not used for comparative analysis due to greater variation as subjects became accustomed to their assigned intervention diets. Additionally, the control group completed food

logs for two weeks only, thus comparisons between intervention groups and the control group necessitated utilization of food logs completed during the two weeks between baseline and follow-up testing. Regardless, analysis of food logs during the familiarization period and the intervention period for subjects engaged in training showed no significant differences between these two time periods.

The decreased caloric intake in the low protein group reflected the difficulty in convincing subjects to replace protein sources with greater carbohydrate intake. While there were no significant differences in carbohydrate or fat consumed between high and low protein groups, the reduction of calories from protein resulted in lower overall caloric intake for the low protein groups. Despite the fact that the low protein diet was also lower in calories, there were no detrimental effects on performance measures, body composition, or rates of muscle protein synthesis.

Interestingly, there were no differences in any measure of lean mass or lean mass change in total body, total thigh, or thigh cross section among groups. This result suggests that multiple strategies for maintaining and/or accreting muscle mass may be utilized depending on the level of dietary protein intake. The conclusion that ingesting a 1.3±0.3 g/kg/day protein diet, considered "low" compared to recommended protein intakes and actual amounts of protein eaten by athletes, coupled with three-hour delayed nutrient ingestion post-exercise did not negatively affect muscle mass is at odds with the high protein, post-exercise supplementation dogma that would predict an increase in protein degradation and decrease in muscle anabolism in this condition based upon studies individually examining protein timing and total protein consumption.

While the LO/DPE group had a higher thigh fat mass at baseline, their average fat mass at follow-up was similar which resulted in a greater decrease in fat mass from baseline to follow-up. It is possible that the lower caloric intake of the LO/DPE group caused greater utilization of fat stores for energy while preserving lean mass. On the other hand, groups with higher protein intakes may have converted excess amino acids to energy substrate, preventing the need to tap into fat stores. Previous studies have proposed the "muscle full" hypothesis that once a maximum amount of amino acids is delivered to the muscle, any additional amino acids will be oxidized for energy (6, 10a, 76a). This strategy may not be advantageous, however, and potential drawbacks of excess nitrogen in the diet will be discussed further.

While normalized isokinetic knee extension strength was significantly greater at baseline in the LO/IPE and HI/DPE groups compared to controls, there were no other significant differences at follow-up or in percent change for any strength and power measures that were normalized to thigh lean mass. Our tests of 1RM did show greater absolute strength in knee extension at follow up in LO/DPE compared to controls, and a greater percent change in knee extension 1RM in HI/DPE compared to controls. It is possible that there was some sort of learning effect associated with the knee extension machine, since multiple groups showed a significant increase over controls who did not use the machine regularly. While both legs were exercised simultaneously on the knee extension machine, the unilateral design allowed each leg to move independently and therefore isolated each individual quadricep muscle. At the time of baseline testing, subjects in the intervention groups would have used the machine for two weeks during the familiarization period, potentially giving them an advantage over the control group. This exercise is likely the most applicable exercise due to the isolation of the muscle that was sampled in biopsies, yet there were no differences in 1RM strength among intervention groups,

suggesting yet again that total and timing of protein intake did not differentially affect performance measures.

Discussion

As previously discussed in the Introduction, many factors could have influenced results of previous studies that found increased rates of protein synthesis with high levels of protein ingestion and protein supplementation immediately post-exercise. The use of untrained subjects and/or subjects not yet adapted to the prescribed exercise stimulus could have caused an exaggerated response to exercise and protein ingestion. Untrained subjects have been shown to exhibit elevated protein requirements during the adaptation period (60, 61, 102). However, this condition does not describe the athletic population to which resulting recommendations have been applied. This study not only utilized subjects with a uniform, habitual training background, but also allowed ample time for subjects to reach a steady-state within the training protocol.

Use of fixed label isotopic tracers that require fasting prior to and resting during infusion may alter the metabolic state post-exercise and thus affect rates of protein synthesis. Even with fasting, assumptions regarding the precursor pool of the label used are likely incorrect (40). Additionally, the assumptions of tracer behavior may not hold with exercise (40, 116), which is a key component to the question at hand. Due to both fasting and resting requirements, the window of measurement with fixed label isotopic tracers has remained small, typically 1-3 hours (40). However, individual responses to feeding and exercise vary, necessitating a larger measurement period to observe the cumulative effect of these stimuli. Previous studies that have shown an increase in rates of protein synthesis without subsequent increases in lean mass may thus be confounded by the fixed label methodology used to measure MPS (3, 34, 53, 82). Furthermore, both fasting and exercise result in increases in MPB which could have affected

previous studies' conclusions regarding timing of protein supplementation. If subjects were already fasted prior to the exercise and nutrition intervention as a requirement for fixed label isotopic tracer methods, then subjects receiving their supplement at a later time point post-exercise would experience a longer period of net negative amino acid balance. This extended period of MPB caused by methodological limitations could create the appearance of a detrimental of effect of delayed supplementation compared to immediate supplementation.

Conversely, measurement of FSR with the deuterium oxide label does not require fasting, resting, or assumptions regarding a precursor pool. Therefore, the subject's metabolism, specifically amino acid metabolism, is not impacted and the deuterium molecule will continue to label nutrients ingested throughout the day at the same proportion to the deuterium pool as it relates to the total body water pool. Additionally, utilization of the deuterium tracer allows labeling of amino acids to occur as proteins are degraded and the resulting free amino acids are recycled for new protein synthesis. When degraded proteins enter the free amino acid pool, they can be labeled by deuterium before reincorporation into muscle tissue. Therefore, the results of our study may differ from previous results due the ability of the deuterium method to account for variation in recycling efficiency.

There are two main interpretations of our results: (1) that the low protein and delayed supplementation groups have an increase in efficiency and amino acid recycling, and/or (2) that the high protein and immediate supplementation groups have a decrease in efficiency and amino acid recycling. If muscle becomes more efficient at recycling amino acids in the lower protein (here, 1.3 g/kg/day) and delayed supplementation condition, cells may become more metabolically active and could exhibit higher protein synthesis rates while maintaining or accreting the same amount of lean mass as in the high protein and immediate supplementation

condition. In a recent study of 43 untrained college-aged males engaged in 12 weeks of resistance training, supplementation with a maltodextrin placebo resulted in greater amino acid transporter activity (LAT1: L-amino acid transporter-1) than groups supplementing with leucine and whey but observed no difference among groups in muscle fiber cross sectional area (98aa). It is therefore possible that a lower protein diet or delayed supplementation metabolically sensitizes muscle cells to respond to protein intake and exercise to a greater extent and with greater efficiency. It is also possible that maximal protein synthesis rates are reached 2-4 hours post-exercise and timing supplementation during this window results in more optimal nutrient uptake. As previously discussed, the dedication of ATP to work output during and for some time after exercise likely results in a dampened MPS response. Therefore, timing protein supplementation three hours post-exercise when protein synthesis rates are increased may have resulted in greater incorporation of ingested protein and tracer into muscle over the 24-hour measurement period. As the primary energy source during exercise, carbohydrate replenishment post-exercise may be a more optimal supplementation strategy. The delayed protein groups may have performed better had carbohydrates been ingested during the three-hour window post-exercise instead of fasting until protein consumption.

Alternatively, the decreased efficiency in groups ingesting high levels of protein and supplementing immediately post-exercise could be responsible for the similarities in MPS among groups in our study. A previous study utilizing a crossover design fed 12 young males either moderate (1.08-1.18 g/kg/day) or high (1.74-2.00 g/kg/day) protein intake and found greater nitrogen excretion in urine and feces and decreased biologic value and net protein utilization when protein intake was high (50aa). This evidence together with the observed decrease in amino acid transporters during whey and leucine supplementation vs. carbohydrate placebo

(98aa) and the "muscle full" hypothesis (6, 10a, 76a) both previously discussed suggest that the high protein/supplemented condition does not result in optimal skeletal muscle protein metabolism. The results of our study additionally indicate that no advantage is gained in strength, power, or lean mass by higher protein intakes or immediate supplementation post-exercise in trained young males.

Applications

Based on this study, there does not appear to be a need for athletes or active individuals engaged in regular training to eat a high protein diet above 1.3 g/kg total mass/day (1.9 g/kg lean mass/day). There was no difference between rates of protein synthesis or lean mass accretion in training groups who ingested 1.3 g/kg protein per day vs. group who ingested 2.2 g/kg/day (2.7 g/kg lean mass/day), nor were there functional performance differences in strength or power. Previous studies have proposed that high protein intakes may not only increase overall calorie intake but also limit the intake of carbohydrates needed to fuel muscle contraction. Carbohydrate intake in this study was not significantly different between high and low groups in this study, however, indicating that individuals who decreased their protein intake to match study requirements did not replace these calories with added carbohydrate or fat. It is important to note that although study participants were persistently reminded and encouraged to replace protein with carbohydrate in the diet by study staff as food logs were evaluated daily, this did not occur to the extent desired perhaps due to previously established dogmas. Thus, the results of this study suggest a reevaluation of nutritional guidance given to athletes and active individuals to emphasize a balanced diet that more closely follows nutritional guidelines and places less emphasis on protein sources. Interestingly, despite the decrease in overall energy, low protein intake groups did not have a significant difference in total lean mass or lean mass accretion in the

body as a whole or in the targeted thigh regions, indicating that individuals were able to adapt to this nutritional strategy.

Protein sources are costly both financially and metabolically due to nitrogen production and urea excretion, potential kidney stress, and dehydration. While effects on kidney function has been suggested as a drawback for high protein diets, there is currently no evidence for adverse effects on the kidneys at this time. A study by Poortmans & Dellalieux (91) showed that individuals eating up to 2.8 g/kg/d protein had higher plasma concentrations of uric acid and calcium but normal renal clearances of creatinine, urea, and albumin. However, very high protein diets have not been studied over long periods of time. Some high protein diets advocate intakes up to 5 g/kg/day (8a), and studies of actual protein consumption in athletes indicates protein intake of 2-3 g/kg/day on average (110). Protein intake on the order of 5 g/kg/day and greater than 35% of calories can lead to hyperaminoacidemia, hyperammonemia, hyperinsulinemia, nausea, and diarrhea (8a). A primary goal for athletes is consuming the optimal diet for performance and obtaining higher efficiency of nutrient utilization. A low or moderate protein diet would not only be more nutritionally efficient, but more affordable as well. Many athletes seeking to reach the elite levels of their sport are likely be financially challenged, often due to the time constraints placed upon them by their training. Maintaining a high protein diet appears to conflict with both financial and metabolic costs without providing additional performance benefits.

Limitations and Delimitations

Limitations and delimitations of this research are important to recognize as they affect the application of study results.

Limitations

The results of this study are subject to certain limitations. Self-reporting measures were used for sleep, activity, food, and training history. While real-time monitoring of these measures would increase confidence in the data, non-compliance with study requirements would not have been expected partly because there was no incentive to mislead investigators. A more thorough study could be conducted in a metabolic unit to verify these measures.

Self-reporting training history could have resulted in individuals overstating the time they had previously trained so that they could participate in the study. However, due to the intensity of the prescribed exercise, a truly untrained individual would not have been able to complete the exercise protocol (and indeed, some did withdraw from the study due to inability to perform the protocol). With two weeks of familiarization prior to the intervention period, all subjects should have reached a uniform training level.

Although the objective of the study was to determine the optimal protein intake for athletes, only moderate and relatively high levels of protein ingestion were tested in this study. Therefore, it is possible that an even lower protein intake could be optimal.

The timing of supplementation focused specifically on protein ingestion, therefore no other nutrients were ingested post-exercise. Thus, the delayed protein group underwent a period of complete fasting post-exercise that was not experienced by the immediately supplemented group. This increased period of muscle protein breakdown could have affected their cumulative muscle protein synthesis.

Finally, the application of these results is limited to healthy, young adult males who are currently in the trained state. While qualitatively similar results would be predicted for healthy, young females, quantitative statements regarding their daily protein requirements and timing of supplementation cannot be made with certainty. Additionally, these results may not be applicable to adolescents or older athletes/active individuals. It is possible that an older population could have higher protein requirements due to an age-related reduction in their ability to adapt to the exercise stimulus by increasing efficiency of amino acid transport, recycling, and utilization. Likewise, this study does not have application to the general population (whose needs are characterized by the RDA), obese individuals, or individuals with chronic illnesses.

Delimitations

The specific aim of this study was to determine the effects of low and high protein intake, immediate and delayed supplementation, and any interaction between these two variables in athletes. Since athletes are a young, healthy population, individuals with chronic illnesses and metabolic dysfunction were excluded, as were individuals greater than 29 years old. To maintain a more uniform population, females and first semester freshmen were excluded due to the variability they would contribute.

While the study required individuals to have a history of aerobic training, no measures of cardiovascular fitness were taken. Since the goal of the study was to determine whether protein intake was indeed elevated in athletes, the strength and power population for which current daily protein recommendations are highest was targeted. The sprint intervals and high intensity resistance training protocol utilized in this study

intended to maximize the exercise stress applied to the muscle, therefore, differences in protein requirements should be greatest and most easily detected in the strength and power trained state. Because the study focus was on muscle anabolism, we chose to measure strength and power performance outcomes instead of cardiovascular and endurance outcomes.

Finally, a control group served as a baseline for the intervention group subjects. Subjects were not their own control in this study for a variety of reasons. Additional pre-study muscle biopsies would have incurred greater up-front cost to investigators and risk to study participants. Most importantly, however, pre-study measures of muscle protein synthesis would have caused deuterium enrichment to persist throughout the familiarization and intervention periods, thus affecting enrichment during post-study measurements of cumulative muscle protein synthesis. Therefore, utilizing a control group with verified similarity to the intervention group was the ideal choice both practically and methodologically.

Summary

The objectives of this study were to determine (1) whether steady-state trained individuals have elevated total protein requirements, (2) whether immediate post exercise protein intake affects cumulative muscle protein synthesis and total lean mass, and (3) whether there is an interaction between timing of protein intake post-exercise and total daily intake on anabolic muscle responses. In a double blind, randomized controlled trial with 45 healthy, previously-trained young men, we determined that total protein requirements were not elevated above 1.3 g/kg total mass/day, or 1.9 g/kg lean

mass/day, nor did timing of protein intake affect the primary outcome of muscle protein synthesis or secondary outcomes of body composition, strength, and power. In fact, only the group consuming low daily protein and supplementing three hours delayed post-exercise showed a significantly higher FSR compared to controls. Thus, our hypothesis that individuals undergoing consistent training have adapted to their workload and increases in daily protein intake would not have additive effects on cumulative muscle protein synthesis was not rejected. Similarly, we were unable to reject the hypothesis that cumulative muscle protein synthesis would not be significantly different between immediate and delayed post-exercise protein supplementation. Finally, the hypothesis that there would be no interaction between total daily protein requirements and timing of protein supplementation post-exercise was also not rejected. This study only compared a modest (1.3 g/kg/day) and a relatively high (2.2 g/kg/day) protein intake. Future studies are necessary to determine whether the lower threshold for protein intake is different from the RDA of 0.8 g/kg/day, or if previously trained individuals in a steady-state training condition are able to adapt to such a protein intake. Furthermore, we call for future recommendations to be given relative to lean mass to reduce the overconsumption of protein in athletes with higher body fat. Additional studies incorporating carbohydrate supplementation post-exercise would be beneficial for comparison to post-exercise protein supplementation. In our study, the lower protein, three-hour delayed group was able to optimize their efficiency to maintain high performance measures and rates of muscle protein synthesis, but it is unclear if they could have performed better had they ingested additional calories from carbohydrates post-exercise rather than

fasting. Future studies should also investigate cell signaling by analyzing the cytosolic portion of muscle samples with Western Blots to determine the metabolic activity of cells under varying total protein intakes and supplementation schemes. Such studies would provide insight into the cellular mechanisms that led to the results presented here. Finally, future studies should also further investigate additional effects of long-term high protein intake, including but not limited to hydration status, glycogen storage and utilization, excess nitrogen and nitric oxide production, and amino acid transport and utilization within the muscle. Such studies would allow a more accurate characterization of the optimal amount and timing of protein ingestion for well-trained athletes.

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APPENDIX A

1. Consent Form

Project Title: ***Revisiting total protein intake and timing of ingestion in conjunction with exercise utilizing deuterium***

You are invited to take part in a research study being conducted by Steven Riechman (Principle Investigator), Erin Simmons (Co-principle Investigator), and James Fluckey (Co-principle Investigator), researchers from Texas A&M University and funded by the Huffines Institute. The information in this form is provided to help you decide whether or not to take part. If you decide to take part in the study, you will be asked to sign this consent form. If you decide you do not want to participate, there will be no penalty to you, and you will not lose any benefits you normally would have.

Why Is This Study Being Done?
The purpose of this study is to clarify the total protein ingestion requirements for active individuals and athletes who are involved in stable exercise training, and to determine whether there is an optimal time to ingest protein following exercise events.

Why Am I Being Asked To Be In This Study?
You are being asked to be in this study because you are a healthy male, aged 19-29 years old, who has been involved in both strength training and cardiovascular exercise for over one (1) year. As a Corps of Cadets member, you are being asked to participate in the study to lessen the potential for variability between study participants (in terms of food, sleep, schedule, training history, etc.). You have also been selected because you have informed researchers that you:

1. Do not have any metabolic disorders including known electrolyte abnormalities, heart disease, arrhythmias, diabetes, thyroid disease, or hypogonadism;
2. Do not have a history of hypertension, hepatorenal, musculoskeletal, autoimmune, or neurologic disease;
3. Do not have any bleeding disorders and are not taking any anti-coagulants;
4. Are not taking thyroid, hypoglycemic, anti-hypertensive, or androgenic medications;
5. Have not taken ergogenic levels of nutritional supplements that may affect muscle mass (e.g. HMB, creatine), insulin-like substances, or anabolic/catabolic hormone levels (DHEA, etc.) within six months prior to the start of the study.

How Many People Will Be Asked To Be In This Study?
One hundred (100) people (participants) will be invited to participate in this study.

What Are the Alternatives to Being in This Study?
The alternative to being in the study is not to participate.

What Will I Be Asked To Do In This Study?
If you agree to participate in this study, you will report to the laboratory four (4) days per week for two (2) weeks to familiarize you with the exercise protocol. You will then undergo baseline testing, starting with a dual energy x-ray absorptiometry (DEXA) scan to measure body composition after the familiarization period and before the start of the study. You will also be asked to utilize an isokinetic dynamometer to obtain a measure of your maximal voluntary contraction. You will be asked to pedal on a power bike to obtain measures of maximal rate of force development, peak power, and relative power.

You will be asked to attend one nutrition education session and to follow a well-balanced diet that meets certain daily protein requirements, and document all your food intakes using a program (Nutribase) that will be installed on your PC. Additionally, you will be asked to log activity each day.

If you are selected for an intervention group, you will be asked to perform cycling sprint intervals and resisted leg extensions as part of the exercise routine for four days each week. Trained personnel will supervise your training sessions. Each exercise session will take approximately 90 minutes. You will consume a protein supplement either immediately following each exercise session or three (3) hours post-exercise.

Before and after your final exercise session, blood samples will be collected from your arm (6 blood draws total). You will also be asked to consume four (4) boluses of deuterium oxide tracer following the final exercise session.

You will also be asked to provide a muscle sample from your leg muscle for us to determine muscle protein synthesis rates. Muscle biopsies will be conducted immediately before the final exercise session, and again 24 hours after the final exercise session. Samples will be obtained using the Bergstrom technique, which involves a 1 cm incision on the skin and the use of a 5mm biopsy needle using sterile procedures. Local anesthetic will be used prior to incision and biopsy. Percutaneous muscle biopsies (50-70 mg) will be obtained from the middle portion of the *vastus lateralis* muscle (thigh muscle covering the outermost portion of the front of the leg) of one leg at the midpoint between the knee and hip joint at a depth between 2 and 4 cm. For the final biopsy, the procedure will be repeated about 2 cm closer to the hip joint.

Your participation in this study will last up to fifty-five (55) hours over the course of twenty-nine (29) days (approximately 4 weeks) and includes twenty-two (22) visits.

You may be removed from the study by the investigator for these reasons:

1. Excessive use of alcohol
2. Performance of exercise outside of this study
3. Use of medication that increases bleeding risks
4. Injury that results in the inability to perform study tasks

Are There Any Risks To Me?

This study will involve minor increases above minimal risk that are greater than those you would come across in everyday life.

1. Sprint interval and resistance exercise may lead to discomfort, pain, and muscle injury. These risks are reduced by inclusion of appropriate warm-ups and stretches, proper performance of exercises, and discontinuation of exercise if necessary. The exercise sessions will be closely supervised by trained personnel to ensure appropriate compliance.
2. The biopsy procedure carries the risk of complications including soreness (100%), infection (<1%), and permanent numbness («1%). Additional risks include discomfort, bleeding and possible scarring at biopsy site.
3. Risks associated with blood sampling include minor discomfort at puncture site and possible bruising. There is a slight risk of infection, however, only trained phlebotomist will be performing blood sampling using previously approved sterilization procedures.
4. DEXA scan for body composition measurement uses low amounts of radiation (less than a 2 hour plane ride).
5. Ingestion of deuterium oxide can cause lightheadedness, dizziness, and nausea if consumed quickly, however, consumption will be monitored to reduce the risks of these effects. No other side effects for deuterium have been observed.
6. There is potential economic risk for medical referral if needed.

Are There Any Benefits To Me?

The direct benefits to you by being in this study include:

1. Receipt of body composition and bone density data from DEXA analysis as well as information from strength and power tests.
2. Provision of protein supplements.
3. Provision of training with professional trainers.
4. Participants will be able to access nutritional software and will be provided with a copy of their dietary analysis.

NOTE: If you are randomly selected to be part of the control group, you will not receive supplements or training, and time commitments will be significantly decreased.

Will There Be Any Costs To Me?

Aside from your time, there are no costs for taking part in the study.

Will I Have To Pay Anything If I Get Hurt In This Study?

If you are hurt in this study, it will be your responsibility to notify the study investigators, to seek treatment, and to pay for such treatment. You will be recommended to Beutel Health Center on the Texas A&M Campus, or you may elect to be treated by your preferred physician.

If you suffer any injury as a result of taking part in this research study, please understand that nothing has been arranged to provide free treatment of the injury or any other type of payment. However, all needed facilities, emergency treatment and professional services will be available to you, just as they are to the community in general. You should report any injury to Dr. Steven Riechman at (979) 862-3213. You will not give up any of your legal rights by signing this consent form.

Side effects (injury) can happen in any research study. These effects may not be your fault or the fault of the researcher involved. Known side effects have been described in the "Are there any risks to me?" section of this consent form. However, side effects that are not currently known may happen and require care. You do not give up any of your legal rights by signing this form.

Will I Be Paid To Be In This Study?
You will receive $100 for completing the study. Disbursement will occur following the final muscle biopsy. Partial payment of $50 may occur if only one biopsy is performed. No compensation will be provided before the completion of the first biopsy.

Will Information From This Study Be Kept Private?
The records of this study will be kept private. No identifiers linking you to this study will be included in any sort of report that might be published. Research records will be stored securely and only principle and co-principle investigators Steven Riechman, Erin Simmons, and James Fluckey will have access to the records. You will be given an identifying code during this study, and the key to decode data will be stored separately.

Information about you will be stored in a locked file cabinet in a locked laboratory, and computer files will be protected with a password and stored on a secure server. This consent form will be filed securely in an official area.

People who have access to your information include the Principal Investigator and research study personnel. Representatives of regulatory agencies such as the Office of Human Research Protections (OHRP) and entities such as the Texas A&M University Human Subjects Protection Program may access your records to make sure the study is being run correctly and that information is collected properly.

Information about you and related to this study will be kept confidential to the extent permitted or required by law. If there are any reports about this study, your name will not be in them.

Who may I Contact for More Information?
You may contact the Principal Investigator, Steven Riechman, Ph.D., to tell him about a concern or complaint about this research at 979-862-3213 or sriechman@hlkn.tamu.edu. You may also contact the Co-Principle Investigator, Erin Simmons, M.S., at 940-300-6029 or ees06f@tamu.edu.

For questions about your rights as a research participant, to provide input regarding research, or if you have questions, complaints, or concerns about the research, you may call the Texas A&M University Human Subjects Protection Program office by phone at 1-979-458-4067, toll free at 1-855-795-8636, or by email at irb@tamu.edu.

What if I Change My Mind About Participating?
This research is voluntary and you have the choice whether or not to be in this research study. You may decide to not begin or to stop participating at any time. If you choose not to be in this study or stop being in the study, there will be no effect on your student status, medical care, employment, evaluation, relationship with Texas A&M University, etc. Any new information discovered about the research will be provided to you. This information could affect your willingness to continue your participation.

STATEMENT OF CONSENT
I agree to be in this study and know that I am not giving up any legal rights by signing this form. The procedures, risks, and benefits have been explained to me, and my questions have been answered. I know that new information about this research study will be provided to me as it becomes available and that the researcher will tell me if I must be removed from the study. I can ask more questions if I want at any time. A copy of this entire consent form will be given to me.

_____ _____
Participant's Signature Date

_____ _____ _____
Printed Name Date

INVESTIGATOR'S AFFIDAVIT:
Either I have or my agent has carefully explained to the participant the nature of the above project. I hereby certify that to the best of my knowledge the person who signed

this consent form was informed of the nature, demands, benefits, and risks involved in his/her participation.

_____ _____

Signature of Presenter Date

_____ _____

Printed Name Date

2. Consent to Provide PHI

Project Title: Revisiting total protein intake and timing of ingestion in conjunction with exercise utilizing deuterium

The federal and state governments have issued a privacy rule to protect the privacy rights of individuals enrolled in research. The privacy rule is designed to protect the confidentiality of an individual's health information. This describes your rights and explains how your health information will be used and disclosed for this study.

PURPOSE

You are being invited to participate voluntarily in the above-titled research project. The purpose of collecting Protected Health Information (PHI) for this study is help researchers answer the questions that are being asked in this research study.

WHAT INFORMATION MAY BE USED AND GIVEN TO OTHERS?

Information that will be collected about you includes:

- Age
- Height
- Weight
- Medical history
- Training history
- Body composition
- Maximal voluntary contraction
- Peak power output
- Maximal rate of force development
- Relative power
- Food records
- Muscle protein synthesis rates

WHO MAY USE AND RECEIVE INFORMATION ABOUT ME?

Information about you may be given out by the Principal Investigator and study personnel to:

- Representatives of regulatory agencies (including Texas A&M University Human Subjects Protection Program) to ensure quality of data and study conduct.

WHY WILL THIS INFORMATION BE USED AND/OR GIVEN TO OTHERS?

This information will be used to more accurately characterize the role of protein ingestion in stimulating muscle protein synthesis in exercising individuals and athletes. Your information may be given to regulatory agencies to ensure compliance with all human subjects research guidelines.

The results of this research may be published in scientific journals or presented at professional meetings, but your identity will not be revealed.

HOW LONG WILL THIS INFORMATION BE USED AND/OR GIVEN TO OTHERS?
Your PHI will be linked to your identifying information for two years. After this time, all links will be destroyed and your identity will not be able to be determined.

This authorization will expire on the date the research study ends.

MAY I REVIEW OR COPY THE INFORMATION OBTAINED FROM ME OR CREATED ABOUT ME?
You have the right to access your PHI that may be created during this study as it relates to your treatment or payment. Your access to this information will become available only after the study analyses are complete.

MAY I WITHDRAW OR REVOKE (CANCEL) MY PERMISSION?
You may withdraw this authorization at any time by notifying the Principal Investigator in writing. If you choose to withdraw your authorization, any information previously disclosed cannot be withdrawn and may continue to be used. The address for the Principal Investigator is 213E Heldenfels Hall, Texas A&M University, College Station, Texas, 77840.

WHAT IF I DECIDE NOT TO GIVE PERMISSION TO USE AND GIVE OUT MY HEALTH INFORMATION?
You may refuse to sign this authorization form. If you choose not to sign this form, you cannot participate in the research study. Refusing to sign will not affect your present or future medical care and will not cause any loss of benefits to which you are otherwise entitled.

IS MY HEALTH INFORMATION PROTECTED AFTER IT HAS BEEN GIVEN TO OTHERS?
Once information about you is disclosed in accordance with this authorization, the individual or organization that receives this may redisclose it and your information may no longer be protected by Federal Privacy Regulations.

CONTACTS
You can obtain further information from the Principal Investigator, Steven Riechman, Ph.D., at (979) 862-3213 or sriechman@hlkn.tamu.edu. You may also contact the Co-Principle Investigator, Erin Simmons, M.S., Ph.D. candidate, at 940-300-6029 or ees06f@tamu.edu. If you have questions concerning your rights as a research subject, you may call the Human Subjects Protection Program office at 979-458-4067 or via email at irb@tamu.edu.

AUTHORIZATION

I hereby authorize the use and disclosure of my individually identifiable health information. I will be given a copy of this signed authorization form.

_____ _____

Subject's Signature Date

_____

Printed Name of Subject

_____ _____

Signature of Subject's Legal Representative (if necessary) Date

_____

Printed Name of Subject's Legal Representative

_____

Relationship to the Subject

3. Medical History Questionnaire

Questionnaire

Project Title: ***Revisiting total protein intake and timing of ingestion in conjunction with exercise utilizing deuterium***

Name: _____
Age: _____
Sex (circle one): Male / Female

How long have you been engaged in resistance exercise/strength training?
_____

Do you have any metabolic disorders including known electrolyte abnormalities, heart disease, arrhythmias, diabetes, thyroid disease, or hypogonadism? (circle one) Yes / No

Do you have a history of hypertension, hepatorenal, musculoskeletal, autoimmune, or neurologic disease? (circle one) Yes / No

Do you have any bleeding disorders? (circle one) Yes / No

Are you taking thyroid, hypoglycemic, anti-hypertensive, or androgenic medications? (circle one) Yes / No

Have you taken ergogenic levels of nutritional supplements that may affect muscle mass (e.g. HMB, creatine), insulin-like substances, or anabolic/catabolic hormone levels (DHEA, etc.) within six months prior to the start of the study? (circle one) Yes / No

If you answered yes to the above question, please list any supplements you have taken within the past 6 months:

_____

_____

_____

Please list the medications you are currently taking or have taken in the past 6 months:

_____

_____

_____

4. List of Medications that Increase Bleeding Risk

Drugs That Increase Bleeding

The following list of drugs includes many, but not all, drugs that can impair normal clotting mechanisms. For example Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), such as aspirin and ibuprofen, bind to blood platelets and impair platelet during blood coagulation. Red wine and vitamin E also impair clotting. Coumadin (warfarin), which impairs clotting by competing with vitamin K, is given to patients who have a illness that causes excessive clotting. It is important that these drugs be avoided before surgical procedures.

Advil
Aleve
Alcohol
Alka Seltzer
Amigesic
Anacin
Anaprox
Anaproxin
Ansaid
APC
Argesic
Arthra G
Arthropan
A.S.A.
Ascodeen
Ascriptin
Aspergum
Aspirin
BC Powder
Baby Aspirin
Bayer
Brufen
Bufferin
Butazolidin
Cephalgesic
Cheracol Caps
Children's Aspirin
choline salicylate
Clinoril
Congesprin
Cope
Coricidin
corticosteroids
Coumadin
Darvon ASA
Darvon Compound
Daypro
Depakote
dexamethasone
diclofenac
dipyridamole
Disalcid
divalproex
Doan's Pills
Dolobid
Dristan
Easprin
Ecotrin
Empirin
Emprazil
Endodan
Excedrin
Feldene
fenoprofen
feverfew
Fiorinal
flurbiprofen
Froben
4-Way Cold Tabs
Garlic Capsules
Gelpirin
Genpril
Genprin
Ginko Biloba
Goody's Body Pain
Haltran
Halfprin
Ibuprin
ibuprofen
Ibuprohm
Indameth
Indocin
indomethacin
ketoprofen
ketorolac
Lortab ASA
Magan
Mg sallicylate
meclofenamate
Meclofen
Medipren
mefenamic
Menadob
Midol
Mobidin
Monogesic
Motrin
nabumetone
Nalfon
Naprosyn
naproxen
Norgesic
Norwich Ex.Str.
Nuprin
Ocufen
Orudis
Oruvail
oxyphenbutazone
Oxybutazone
oxyprozin
Pamprin
Pepto-bismal
Percodan
Persantine
Phenaphen
Phenylbutazone
piroxicam
Ponstel
Prednisone
Quagesic
Red Wine
Relafen
Rexolate
Robasissal
Roxiprin
Rufin
Saleto
Salflex
salsalate
Salsitab
Sine Off
Sine Aid
Na thiosalicylate
Soma Compound
sulindac
Synalgos DC
Tanacetum parthenium= (feverfew)
Tolectin
tolmetin
Toradol
Trandate
Trendan
Trental
Trigesic
Trilisate
Tusal
Vanquish
Vitamin E
Voltaren
Warfarin
willow bark
Zactrin
Zorprin

5. Information for Subjects

Information for Prospective Participants:

We are currently recruiting research participants for a study to be conducted within the Department of Health & Kinesiology at Texas A&M University.
The duration of the study will be approximately 4 weeks, which includes 2 weeks of familiarization with the exercise procedures followed by 2 weeks of the experimental period. You will receive $100 at the completion of all the study procedures.

The purpose of the study is to investigate the effects of protein supplementation on muscle metabolism.

What is required?

1. You will be required to perform 16 sessions of supervised high intensity sprint interval and resistance exercises (~80 minutes per session).
2. You will be required to consume a protein supplement following exercise during the study period.
3. You will be required to keep track of what you eat/drink and may be asked to make changes during the study period so that your dietary protein intake matches study requirements.
4. Six blood samples and two muscle biopsy samples will be taken.
5. The exercise sessions/sample collections/tests are most likely to be conducted between 7-9 am.

Who is eligible?

1. Healthy males between the ages of 19-29 years.
2. Member of the Texas A&M Corps of Cadets.
3. Persons involved in both strength training and cardiovascular exercise for at least one (1) year.
4. You cannot participate in the study if you have any of the following:
    a. A blood pressure > 160/100
    b. Cardiac Arrhythmias
    c. Cancer
    d. Hernia
    e. Aortic Aneurysm
    f. Kidney Disease
    g. Lung Disease
    h. Smoker
    i. Cannot be currently taking cholesterol lowering medications
    j. If you are allergic to egg, milk, soy, peanut, or Lidocaine (a local anesthetic).

Please feel free to email or call if you have any further questions or if you fit the criteria and are interested in signing up. If you are interested, the next step would be to have you come in and go over the details of the study, have you sign an informed consent document, provide information about your medical history/exercise readiness prior to the start of this investigation. Contact Erin Simmons at ees06f@tamu.edu with any additional inquiries.

6. Physical Activity Questionnaire

Modifiable Activity Questionnaire Study ID: _____

Check all activities that you have done more than ten times in the past year. Check the months you did each activity over the past 12 months and then estimate the amount of time spent in each activity.

	Activity	Past 12 Months												Average # of times per month	Average # of Minutes each time
		JAN	Feb	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC		
1	Running (outdoor, treadmill)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
2	Swimming (laps, snorkeling)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐		
3	Bicycling (indoor,outdoor)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
4	Softball/baseball	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐		
5	Volleyball	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
6	Bowling	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
7	Basketball	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
8	Skating (roller, ice, blading)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐		
9	Martial Arts (karate, judo)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
10	Tai Chi	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
11	Calisthenics/Toning exercises	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
12	Football	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
13	☐ Soccer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
14	Racquetball/Handball/Squash	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐		
15	Horseback riding	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
16	Hunting														
17	Fishing	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
18	Aerobic Dance/Step aerobic	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
19	Water Aerobics	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
20	Dancing (Square/Line/Ballrm)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐		
21	Gardening or Yardwork	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
22	Badminton	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐		
23	Strength/Weight training	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
24	Rock climbing	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
25	Scuba Diving	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
26	Stair master	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐		
27	Fencing	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
28	Hiking	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐		
29	Tennis	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
30	Golf	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
31	Canoeing/Rowing/Kayaking	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
32	Water skiing	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐		
33	Snow skiing (X-country/Nordic)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
34	Downhill Skiing	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐		
35	Snow shoeing	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
36	Yoga	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
37	Other	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
38	Walking	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐		

Modifiable Activity Questionnaire

Study ID: ___ ___ ___ ___

date ___ ___ ___ age ___ ___ ___ 0

Check all activities that you have done more than ten times in your lifetime excluding school physical education classes. If you have done the activity more than ten times then estimate the amount of time spent in number of years, months per year and hours per week in each activity for each period of your life.

	Activity	Age Period											
		12-18 yr (7 yr total)			19-34 yr (16 yr total)			35-49 yr (15 yr total)			> 50 yr (__ yr total)		
		no yr	mo/yr	hr/wk	no yr	mo/yr	hr/wk	no yr	mo/yr	hr/wk	no yr	mo/yr	hr/wk
1	Running (outdoor, treadmill)												
2	Swimming (laps, snorkeling)												
3	Bicycling (indoor,outdoor)												
4	Softball/baseball												
5	Volleyball												
6	Bowling												
7	Basketball												
8	Skating (roller, ice, blading)												
9	Martial Arts (karate, judo)												
10	Tai Chi												
11	Calisthenics/Toning exercises												
12	Football ☐												
13	Soccer												
14	Racquetball/Handball/Squash ☐												
15	Horseback riding												
16	Hunting												
17	Fishing												
18	Aerobic Dance/Step aerobic												
19	Water Aerobics												
20	Dancing (Square/Line/Ballrm)												
21	Gardening or Yardwork												
22	Badminton												
23	Strength/Weight training												
24	Rock climbing												
25	Scuba Diving												
26	Stair master												
27	Fencing												
28	Hiking												
29	Tennis												
30	Golf												
31	Canoeing/Rowing/Kayaking												
32	Water skiing												
33	Snow skiing (X-country/Nordic)												
34	Downhill Skiing												
35	Snow shoeing												
36	Yoga												
37	Other												
38	Walking												

In general, how many HOURS per DAY do you ususally spend watching television? ☐ hours /day

Over this past year, have you spent more than one week confined to a bed or chair as a result of an injury, illness, or surgery?

YES ☐ NO ☐

If yes,how many weeks over the past year were you confined to a bed or chair? ☐ week(s)

Do you have difficulty doing any of the following activities?

	YES	NO
a. getting in or out of bed or chair?	☐	☐
b. walking across a small room without resting?	☐	☐
c. walking for ten minutes without resting?	☐	☐

Did you ever compete in an individual or team sport (except any time spent in sports performed during school physical education classes?

YES ☐ NO ☐

If yes, how many total years did you participate in competitive sports? ☐ years

Please describe the highest level of competition you achieved (e.g. 5A high school, Division III college)

Sport	_____	(e.g soccer)
Highest Level	_____	(e.g. 5A high school)
Achievement	_____	(e.g. 3 year starter, 3rd team all-district)
Sport	_____	(e.g. swimming)
Highest Level	_____	(e.g. Division III college)
Achievement	_____	(e.g. conference champion 300M)
Sport	_____	
Highest Level	_____	
Achievement	_____	
Sport	_____	
Highest Level	_____	
Achievement	_____	
Sport	_____	
Highest Level	_____	
Achievement	_____	

7. Corps of Cadets Approval Letter

OFFICE OF THE COMMANDANT
Operations & Training

TEXAS A&M UNIVERSITY

20 May 2016

Ms Erin Simmons, M.S.
Department of Nutrition and Food Science
Texas A&M Universtiy

Subject: Support of Doctoral Thesis of Erin Simmons, M.S., Focusing on Protein Requirements for Active Males

I provide this letter in order to state the intent to provide support to the subject doctoral thesis of Ms Erin Simmons, M.S. The Office of the Commandant will advertise and encourage male cadets between the ages of 20 and 28 to volunteer for the study as outline by Ms Simmons.

This intent to support does not equate to an agreement by this office to provide any or all of the requisite number of study participants. Participation in the study remains a volunteer choice by each cadet. The Office of the Commandant will advertise the concept of the study, provide Ms Simmons an opportunity talk with cadets interested in the study, and encourage cadets to volunteer. This office will also make accommodations in the individual training schedules of the volunteer cadets to meet the requirements of the study.

If additional information, approvals, or other contact is required with this office, I can be contacted at 979-862-4311 or gstarnes@corps.tamu.edu.

Glenn T Starnes
Colonel, USMC (Ret.)
Asst Cmdt Operations & Training

Copy for:
Institutional Review Board (IRB)

8. Corps Dorm Recruitment Flyer

9. Email Recruitment Script

Email Recruitment

Howdy!

My name is Erin Simmons. I am a graduate student working in the Nutrition and Food Science and the Health & Kinesiology departments for the Human Countermeasures and Muscle Biology labs. Currently we are conducting a research study regarding the effect of protein supplementation on muscle metabolism.

The nature of the study will include nutrition education sessions, DEXA scan, blood draws, and four weeks of sprint interval and resistance exercise training. Two muscle biopsies will also be performed at the end of the study, and several other non-invasive measurements will be taken throughout the study. You may be asked to consume a protein supplement after your workouts.

The study will last approximately 29 days. Participation is completely voluntary. This study is not associated with any current classes or your membership in the Corps of Cadets in any manner. You may decide not to participate or to withdraw at any time without your current or future relations with the Corps of Cadets or Texas A&M University being affected. There will be a $100 compensation for completing the study. Disbursement will occur after you finish the last biopsy at the end of the study. This study is strictly confidential, and any personal information will not be released at any point.

If you are interested in participating in the study or would like to find out more information regarding it, please contact me at ees06f@tamu.edu.

10. In-Person Recruitment Script

In-Person Recruitment

Hi, my name is Erin Simmons. I am a graduate student working in the Health & Kinesiology department for the Human Countermeasures and Muscle Biology labs. Currently we are conducting a research study regarding the effect of protein supplementation on muscle metabolism.

The nature of the study will include nutrition education sessions, DEXA scan, blood draws, and four weeks of sprint interval and resistance exercise training. Two muscle biopsies will also be performed at the end of the study, and several other non-invasive measurements will be taken throughout the study. You may be asked to consume a protein supplement after your workouts.

The study will last approximately 29 days. Participation is completely voluntary. This study is not associated with any current classes or your membership in the Corps of Cadets in any manner. You may decide not to participate or to withdraw at any time without your current or future relations with the Corps of Cadets or Texas A&M University being affected. There will be a $100 compensation for completing the study. Disbursement will occur after you finish the last biopsy at the end of the study. This study is strictly confidential, and any personal information will not be released at any point.

If you are interested in participating in the study or would like to find out more information regarding it, please contact me at ees06f@tamu.edu.

11. 24 Hour Physical Activity Recall Form

<u>24 Hour Activity Recall</u>

Project Title: ***Revisiting total protein intake and timing of ingestion in conjunction with exercise utilizing deuterium***

Name: _____

1. In the past 24 hours, did you engage in any strenuous physical activity? (circle one)
Yes No
2. If yes, please explain:
_____
_____
_____
_____

3. Please list <u>ALL</u> activity (including walking to class, marching, drilling, etc.) that you have engaged in over the past 24 hours and how much time you spent on each:

Activity	**Description**	**Time**

12. Stanford Sleepiness Scale

Stanford Sleepiness Scale

This is a quick way to assess how alert you are feeling. If it is during the day when you go about your business, ideally you would want a rating of a one. Take into account that most people have two peak times of alertness daily, at about 9 a.m. and 9 p.m. Alertness wanes to its lowest point at around 3 p.m.; after that it begins to build again. Rate your alertness at different times during the day. If you go below a three when you should be feeling alert, this is an indication that you have a serious sleep debt and you need more sleep.

An Introspective Measure of Sleepiness
The Stanford Sleepiness Scale (SSS)

Degree of Sleepiness	Scale Rating
Feeling active, vital, alert, or wide awake	1
Functioning at high levels, but not at peak; able to concentrate	2
Awake, but relaxed; responsive but not fully alert	3
Somewhat foggy, let down	4
Foggy; losing interest in remaining awake; slowed down	5
Sleepy, woozy, fighting sleep; prefer to lie down	6
No longer fighting sleep, sleep onset soon; having dream-like thoughts	7
Asleep	X

13. Pittsburgh Sleep Quality Index

Sleep Quality Assessment (PSQI)

What is PSQI, and what is it measuring?

The Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep in adults. It differentiates "poor" from "good" sleep quality by measuring seven areas (components): subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month.

INSTRUCTIONS:

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed? _____
2. How long (in minutes) has it taken you to fall asleep each night? _____
3. What time have you usually gotten up in the morning? _____
4. A. How many hours of actual sleep did you get at night? _____
   B. How many hours were you in bed? _____

5. During the past month, how often have you had trouble sleeping because you	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
A. Cannot get to sleep within 30 minutes				
B. Wake up in the middle of the night or early morning				
C. Have to get up to use the bathroom				
D. Cannot breathe comfortably				
E. Cough or snore loudly				
F. Feel too cold				
G. Feel too hot				
H. Have bad dreams				
I. Have pain				
J. Other reason (s), please describe, including how often you have had trouble sleeping because of this reason (s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
9. During the past month, how would you rate your sleep quality overall?	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)

14. OMNI-Res Scale for Ratings of Perceived Exertion

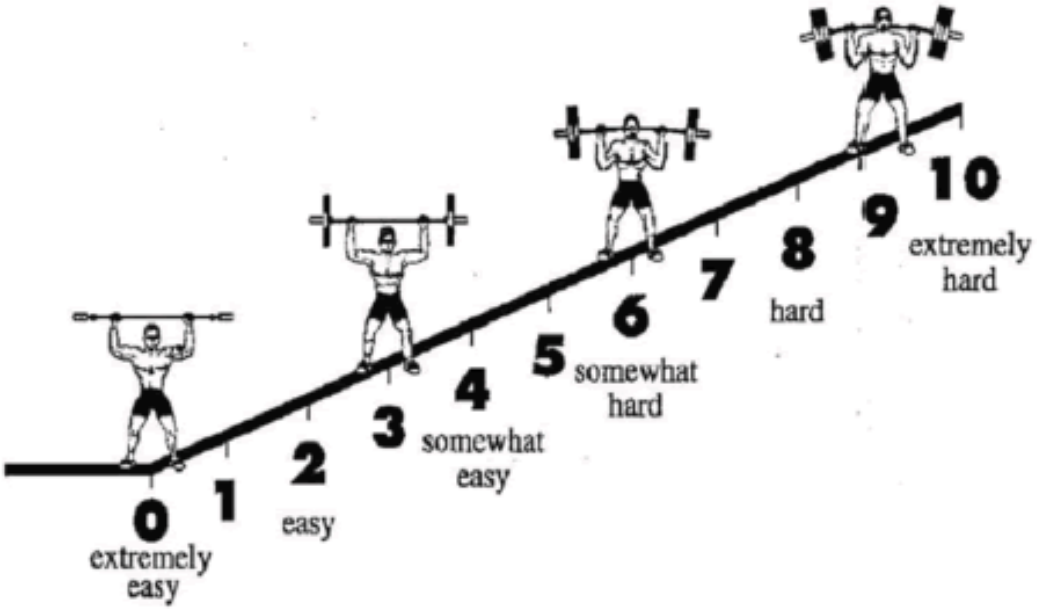

Figure 1: OMNI Perceived Exertion Scale for Resistance Exercise.

APPENDIX B

1. ANOVA Results for Group Demographics

ANOVA Results for Demographics						
		Sum of Squares	df	Mean Square	F	Sig.
Age	Between Groups	29.281	4	7.32	0.712	0.589
	Within Groups	411.451	40	10.286		
	Total	440.732	44			
Height	Between Groups	103.89	4	25.972	0.645	0.634
	Within Groups	1611.296	40	40.282		
	Total	1715.186	44			
Weight	Between Groups	1394.274	4	348.569	1.969	0.117
	Within Groups	7256.877	41	176.997		
	Total	8651.151	45			

Appendix B1 – ANOVA Results for Demographic Characteristics of Five Groups. Means of age (years), height (cm), and weight (kg) were analyzed with a one-way ANOVA for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). There were no significant differences among groups for any measure.

2. ANOVA Results for Overall Modified Activity Questionnaire

ANOVA Results for Overall MAQ						
		Sum of Squares	df	Mean Square	F	Sig.
Past Year hr/wk	Between Groups	160.846	4	40.212	1.319	0.282
	Within Groups	1067.016	35	30.486		
	Total	1227.862	39			
Past Year Met-hr	Between Groups	12427.178	4	3106.795	0.898	0.475
	Within Groups	121025.508	35	3457.872		
	Total	133452.686	39			
Age 12-18 hr/wk	Between Groups	212.836	4	53.209	0.752	0.563
	Within Groups	2546.62	36	70.739		
	Total	2759.456	40			
Age 12-18 Met-hr	Between Groups	10297.308	4	2574.327	0.633	0.642
	Within Groups	146294.819	36	4063.745		
	Total	156592.127	40			
Age 19-34 hr/wk	Between Groups	396.5	4	99.125	1.648	0.187
	Within Groups	1864.434	31	60.143		
	Total	2260.934	35			
Age 19-34 Met-hr	Between Groups	14013.347	4	3503.337	1.375	0.265
	Within Groups	78988.296	31	2548.01		
	Total	93001.643	35			
Lifetime hr/wk	Between Groups	569.802	4	142.451	0.729	0.578
	Within Groups	7226.864	37	195.321		
	Total	7796.667	41			
Lifetime Met-hr	Between Groups	35350.01	4	8837.503	0.967	0.437
	Within Groups	337983.059	37	9134.677		
	Total	373333.069	41			

Appendix B2 – ANOVA Results for Overall Modified Activity Questionnaire. Mean results of the MAQ in hours per week (hr/wk) and Met-hours (Met-hr) were analyzed with a one-way ANOVA for the past year, ages 12-18, ages 19-34, and lifetime for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). There were no significant differences among groups for any measure at any time point.

3. ANOVA Results for Strength Training Modified Activity Questionnaire

ANOVA Results for Strength Training MAQ						
		Sum of Squares	df	Mean Square	F	Sig.
Past Year hr/wk	Between Groups	44.874	4	11.218	1.317	0.283
	Within Groups	298.091	35	8.517		
	Total	342.964	39			
Past Year Met-hr	Between Groups	909.08	4	227.27	1.318	0.282
	Within Groups	6035.184	35	172.434		
	Total	6944.263	39			
Age 12-18 hr/wk	Between Groups	23.328	4	5.832	1.446	0.24
	Within Groups	137.088	34	4.032		
	Total	160.416	38			
Age 12-18 Met-hr	Between Groups	472.773	4	118.193	1.447	0.24
	Within Groups	2777.398	34	81.688		
	Total	3250.171	38			
Age 19-34 hr/wk	Between Groups	49.023	4	12.256	1.543	0.211
	Within Groups	285.982	36	7.944		
	Total	335.005	40			
Age 19-34 Met-hr	Between Groups	992.153	4	248.038	1.542	0.211
	Within Groups	5790.973	36	160.86		
	Total	6783.126	40			
Lifetime hr/wk	Between Groups	119.802	4	29.95	1.686	0.174
	Within Groups	657.08	37	17.759		
	Total	776.882	41			
Lifetime Met-hr	Between Groups	2425.619	4	606.405	1.685	0.174
	Within Groups	13312.097	37	359.786		
	Total	15737.716	41			

Appendix B3 – ANOVA Results for Strength Training Modified Activity Questionnaire. Mean results of the strength training portion of the MAQ in hours per week (hr/wk) and Met-hours (Met-hr) were analyzed with a one-way ANOVA for the past year, ages 12-18, ages 19-34, and lifetime for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). There were no significant differences among groups for any measure at any time point.

4. ANOVA Results for Acute and Chronic Sleep Measures

ANOVA Results for Sleep Variables						
		Sum of Squares	df	Mean Square	F	Sig.
SSS	Between Groups	1.878	3	0.626	0.883	0.46
	Within Groups	22.695	32	0.709		
	Total	24.573	35			
PSQI - Baseline	Between Groups	13.022	4	3.256	0.697	0.599
	Within Groups	186.889	40	4.672		
	Total	199.911	44			
PSQI - Follow-Up	Between Groups	11.497	4	2.874	0.69	0.603
	Within Groups	162.389	39	4.164		
	Total	173.886	43			

Appendix B4 – ANOVA Results for Acute and Chronic Sleep Pattern Measures. Mean acute sleepiness, represented by the Stanford Sleepiness Scale, and mean chronic sleep quality, represented by the Pittsburgh Sleep Quality Index, were analyzed with a one-way ANOVA for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). There were no significant differences among groups for any measure at any time point.

5. ANOVA Results for Low Protein Groups During Familiarization vs. Intervention

ANOVA Results for Low Protein Groups During Familiarization vs. Intervention						
		Sum of Squares	df	Mean Square	F	Sig.
Calories	Between Groups	364816	1	364816	0.933	0.341
	Within Groups	13289604	34	390870.706		
	Total	13654420	35			
Energy(kj)	Between Groups	6539101.361	1	6539101.36	0.954	0.336
	Within Groups	233132255.4	34	6856831.04		
	Total	239671356.8	35			
Protein(g)	Between Groups	821.778	1	821.778	1.243	0.273
	Within Groups	22484.111	34	661.297		
	Total	23305.889	35			
Protein(g/kg TOTAL)	Between Groups	0.124	1	0.124	0.946	0.337
	Within Groups	4.443	34	0.131		
	Total	4.567	35			
Protein(g/kg LEAN)	Between Groups	0.219	1	0.219	1.203	0.28
	Within Groups	6.192	34	0.182		
	Total	6.412	35			
%Calories/Protein	Between Groups	14.694	1	14.694	0.438	0.513
	Within Groups	1141.611	34	33.577		
	Total	1156.306	35			
Carbohydrates(g)	Between Groups	6480.25	1	6480.25	0.858	0.361
	Within Groups	256758.5	34	7551.721		
	Total	263238.75	35			
%Calories/Carbohydrate	Between Groups	11.111	1	11.111	0.13	0.721
	Within Groups	2915.111	34	85.739		
	Total	2926.222	35			
Fat(g)	Between Groups	225	1	225	0.259	0.614
	Within Groups	29532.556	34	868.605		
	Total	29757.556	35			

Appendix B5 – ANOVA Results for Low Protein Group Nutrition During Familiarization and Intervention Periods. Calorie and macronutrient intake during familiarization and intervention periods were analyzed with a one-way ANOVA for the combined low protein groups (LO/DPE and LO/IPE). There were no significant differences between the familiarization and intervention periods for any nutrients.

6. ANOVA Results for High Protein Groups During Familiarization vs. Intervention

ANOVA Results for High Protein Groups FAMILIARIZATION VS. INTERVENTION						
		Sum of Squares	df	Mean Square	F	Sig.
Calories	Between Groups	86534.028	1	86534.028	0.269	0.607
	Within Groups	10921618.28	34	321224.067		
	Total	11008152.31	35			
Energy(kj)	Between Groups	1507984	1	1507984	0.268	0.608
	Within Groups	191067007.6	34	5619617.87		
	Total	192574991.6	35			
Protein(g)	Between Groups	584.028	1	584.028	0.494	0.487
	Within Groups	40194.722	34	1182.198		
	Total	40778.75	35			
Protein(g/kg TOTAL)	Between Groups	0.2	1	0.2	0.844	0.365
	Within Groups	8.074	34	0.237		
	Total	8.275	35			
Protein(g/kg LEAN)	Between Groups	0.106	1	0.106	0.834	0.367
	Within Groups	4.332	34	0.127		
	Total	4.438	35			
%Calories/Protein	Between Groups	2.778	1	2.778	0.139	0.711
	Within Groups	678.222	34	19.948		
	Total	681	35			
Carbohydrates(g)	Between Groups	5852.25	1	5852.25	0.981	0.329
	Within Groups	202740.5	34	5962.956		
	Total	208592.75	35			
%Calories/Carbohydrate	Between Groups	40.111	1	40.111	1.133	0.295
	Within Groups	1203.889	34	35.408		
	Total	1244	35			
Fat(g)	Between Groups	182.25	1	182.25	0.269	0.607
	Within Groups	23048.056	34	677.884		
	Total	23230.306	35			
%Calories/Fat	Between Groups	53.778	1	53.778	2.645	0.113
	Within Groups	691.222	34	20.33		
	Total	745	35			

Appendix B6 – ANOVA Results for High Protein Group Nutrition During Familiarization and Intervention Periods. Calorie and macronutrient intake during familiarization and intervention periods were analyzed with a one-way ANOVA for the combined low protein

groups (HI/DPE and HI/IPE). There were no significant differences between the familiarization and intervention periods for any nutrients.

6a. Nutritional Analysis for Three Groups

Calorie Information		Calories		Energy(kj)		Calories/Protein		%Calories/Protein		Calories/Carbohydrate	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	2031	593	8490	2486	459	90	24	5	882	326
High Protein	18	2655	489	11106	2047	696	127	27	5	1122	274
Control	9	2429	908	10165	3798	534	210	22	4	1017	318
Total	45	2360*	677	9871*	2838	569*	171	25*	5	1005	317
Calorie Information		**%Calories/Carbohydrate**		**Calories/Fat**		**%Calories/Fat**		**Calories/Alcohol**		**%Calories/Alcohol**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	43	9	684	259	33	7	5	19	0.2	0.7
High Protein	18	42	5	826	208	31	5	8	25	0.3	1.0
Control	9	44	9	878	482	35	8	0	0	0	0
Total	45	43	8	780	302	33	6	5	20	0.2	0.8

Proteins		Protein(g)		Protein(g/kg TOTAL)		Protein(g/kg LEAN)		Isoleucine(g)		Leucine(g)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	112	21	1.3	0.3	1.9	0.5	0.2	0.4	0.6	0.6
High Protein	18	170	31	2.2	0.3	2.7	0.5	1.1	1.0	2.1	1.7
Control	9	130	51	1.6	0.7	2.1	0.9	1.9	2.4	3.4	4.2
Total	45	139*	42	1.7*	0.6	2.2*	0.7	0.9*	1.4	1.8*	2.4
Proteins		**Lysine(g)**		**Methionine(g)**		**Phenylalanine(g)**		**Threonine(g)**		**Tryptophan(g)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	0.4	0.6	0.1	0.2	0.3	0.5	0.3	0.8	0	0
High Protein	18	1.9	1.6	0.7	0.7	1.1	1.0	1.0	0.9	0.2	0.4
Control	9	3.1	3.9	0.9	1.3	1.8	2.1	1.8	2.1	0.4	0.7
Total	45	1.6*	2.2	0.5*	0.8	0.9*	1.3	0.9*	1.3	0.2*	0.4
Proteins		**Valine(g)**		**Alanine(g)**		**Arginine(g)**		**Aspartic Acid(g)**		**Cystine(g)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	0.4	0.5	0.3	0.5	0.5	0.6	0.7	0.6	0	0
High Protein	18	1.4	1.1	1.3	1.1	1.6	1.3	2.3	1.7	0.3	0.5
Control	9	2.2	2.6	2.3	2.6	2.6	3.0	3.9	5.1	0.7	1.0
Total	45	1.2*	1.5	1.1*	1.5	1.4*	1.7	2.0*	2.7	0.2*	0.6
Proteins		**Glutamic Acid(g)**		**Glycine(g)**		**Hydroxyproline(g)**		**Proline(g)**		**Serine(g)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	1.6	1.3	0.2	0.4	0	0	0.4	0.5	0.4	0.5
High Protein	18	4.3	3.3	1.2	1.0	0	0	1.4	1.2	1.1	0.9
Control	9	7.9	8.4	1.7	1.8	0.1	0.3	2.3	2.7	2.0	2.7
Total	45	3.9*	4.8	0.9*	1.2	0.02	0.1	1.2*	1.6	1.0*	1.5
Proteins		**Tyrosine(g)**		**Histidine(g)**							
Group	n	Mean	SD	Mean	SD						
Low Protein	18	0.1	0.3	0.1	0.2						
High Protein	18	0.8	0.8	0.8	0.8						
Control	9	1.4	1.8	1.1	1.5						
Total	45	0.7*	1.1	0.6*	0.9						

Carbohydrates		Carbohydrate(g)		Starch(g)		Sugars(g)		Glucose(g)		Fructose(g)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	239	84	8	7	58	40	3.6	6.9	3.4	5.0
High Protein	18	299	73	16	22	77	38	3.5	3.4	4.1	4.3
Control	9	268	83	25	31	78	34	6.6	4.9	6.4	6.3
Total	45	268	83	14	20	70	39	4.2	5.4	4.3	5.0
Carbohydrates		**Galactose(g)**		**Sucrose(g)**		**Lactose(g)**		**Maltose(g)**		**Fiber(g)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	0	0	2.7	3.3	0.1	0.2	0.2	0.4	25	12
High Protein	18	0	0	3.4	2.9	0.7	2.4	0.7	1.0	27	11
Control	9	0	0	4.2	2.4	0.7	1.7	0.4	0.7	20	6
Total	45	0	0	3.3	2.9	0.4	1.7	0.4	0.8	25	11

Fats		Fat(g)		Saturated Fat(g)		TransFat(g)		Trans-Monounsat Fat(g)		Trans-Polyunsat Fat(g)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	74	27	20	8	0.5	0.7	0	0	0	0
High Protein	18	89	22	25	8	0.5	0.6	0	0	0	0
Control	9	95	53	28	16	0.7	1.0	0.1	0.3	0	0
Total	45	84	33	24	10	0.5	0.7	0.02	0.1	0	0
Fats		**Monounsaturated Fat(g)**		**Polyunsaturated Fat(g)**		**Omega3(g)**		**Omega6(g)**			
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Low Protein	18	8.9	6.8	4.2	2.3	0	0	0.8	1.2		
High Protein	18	9.9	5.8	5.7	3.9	0.2	0.7	1.3	1.1		
Control	9	16.6	21.3	8.3	9.6	0.2	0.4	3.2	5.0		
Total	45	10.8	11.0	5.6	5.2	0.1	0.5	1.5	2.5		

Sterols		Cholesterol(mg)		Phytosterol(mg)		Stigmasterol(mg)		Campesterol(mg)		Beta-sitosterol(mg)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	195	68	5	8	0.2	0.4	0.5	0.9	8.4	15.9
High Protein	18	384	180	10	12	0.2	0.5	0.4	0.9	5.9	12.7
Control	9	564	437	12	15	0.3	0.5	0.2	0.4	1.1	1.7
Total	45	345*	262	9	11	0.2	0.5	0.4	0.8	6.0	13.0

Minerals		Calcium(mg)		Chloride(mg)		Magnesium(mg)		Phosphate(mg)		Potassium(mg)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	410	301	2.3	9.9	66	59	226	154	711	492
High Protein	18	568	274	0	0	119	85	456	314	1067	653
Control	9	680	364	0	0	165	133	876	754	1475	1113
Total	45	527	315	0.9	6.3	107*	94	448*	456	1006*	752
Minerals		**Sodium(mg)**		**Chromium(mcg)**		**Copper(mg)**		**Fluoride(mcg)**		**Iodine(mcg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	2901	1014	0.4	1.9	0.1	0.5	114	259	0.7	2.8
High Protein	18	3874	1243	0.2	0.7	0.4	0.6	36	123	0.3	1.4
Control	9	4013	1822	0.3	1.0	0.6	0.7	30	33	0.8	2.0
Total	45	3512*	1362	0.3	1.3	0.3	0.6	66	183	0.6	2.1
Minerals		**Iron(mg)**		**Manganese(mg)**		**Molybdenum(mcg)**		**Selenium(mcg)**		**Zinc(mg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	8.6	4.4	0.6	0.8	0.3	1.2	12	10	3.6	6.0
High Protein	18	10.6	5.7	1.1	1.3	0.2	0.7	43	40	4.5	3.6
Control	9	10.0	5.5	1.4	1.3	0.3	1.0	74	69	6.4	6.0
Total	45	9.7	5.1	1.0	1.1	0.2	1.0	37*	45	4.5	5.2

Other		Alcohol(g)		Ash(g)		Caffeine(mg)		Theobromine(mg)		Choline(mg)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	0.7	2.6	2.7	2.2	20	47	2	5	30	25
High Protein	18	1.2	3.6	4.9	3.7	16	54	3	9	122	130
Control	9	0.0	0.0	8.2	7.1	9	11	25	48	278	406
Total	45	0.8	2.8	4.7*	4.5	16	45	7*	23	116*	213

Other		Betaine(mg)	
Group	n	Mean	SD
Low Protein	18	2	3
High Protein	18	31	67
Control	9	8	9
Total	45	15	44

Vitamins		Vit-A(mcg RAE)		Retinol(mcg)		BetaCarotene(mcg)		AlphaCarotene(mcg)		BetaCryptoxanthin(mcg)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	152	170	53	64	1061	1717	177	404	24	41
High Protein	18	238	238	61	81	1757	2201	283	575	15	27
Control	9	298	259	190	202	1169	1684	147	403	30	65
Total	45	216	221	84*	120	1368	1910	213	473	21	42
Vitamins		**Lycopene(mcg)**		**Lutein+Zeaxanthin(mcg)**		**Vit-A IU**		**Vit-B1(mg)**		**Vit-B2(mg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	300	647	622	1624	3431	3418	0.4	0.5	0.8	2.3
High Protein	18	912	2621	627	1016	4892	3752	0.7	0.7	0.7	0.6
Control	9	394	594	745	876	4435	4030	0.8	0.8	1.3	0.7
Total	45	564	1722	648	1249	4216	3655	0.6	0.6	0.9	1.5
Vitamins		**Vit-B3(mg)**		**Vit-B5(mg)**		**Vit-B6(mg)**		**Folate(mcg)**		**FoodFolate(mcg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	11	17	2.0	5.3	0.9	2.1	118	97	56	77
High Protein	18	13	9	1.9	1.4	0.8	0.8	174	104	86	69
Control	9	14	9	3.1	3.0	1.2	1.0	188	139	113	107
Total	45	12	13	2.2	3.7	1.0	1.4	154	110	79	82
Vitamins		**FolicAcid(mcg)**		**Dietary Folate Equivalent**		**Vit-B12(mcg)**		**Vit-B12, added(mcg)**		**Vit-H(mcg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	29	48	96	96	2.2	6.0	1.1	3.3	1.1	4.7
High Protein	18	35	55	136	127	2.2	3.9	0.9	3.5	0.4	1.9
Control	9	51	46	197	164	3.3	2.8	0	0	0.9	2.7
Total	45	36	50	132	127	2.4	4.6	0.8	3.0	0.8	3.4
Vitamins		**Vit-C(mg)**		**Vit-D(IU)**		**Vit-D2(mcg)**		**Vit-D3(mcg)**		**Vit-D2+D3(mcg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	71	106	35	61	0	0	0.1	0.3	0.2	0.5
High Protein	18	49	41	66	65	0	0	0.7	1.2	0.8	1.2
Control	9	51	32	163	169	0	0	2.3	3.8	2.9	3.8
Total	45	58	73	73*	103	0	0	0.8*	2.0	1.0*	2.1
Vitamins		**AlphaTocopherol(mg)**		**BetaTocopherol(mg)**		**GammaTocopherol(mg)**		**DeltaTocopherol(mg)**		**AlphaTocopherol(mg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	1.6	2.4	0	0	0.6	0.8	0	0	0	0
High Protein	18	1.7	1.3	0.1	0.2	1.4	1.9	0.1	0.3	0.1	0.2
Control	9	3.0	2.4	0.1	0.3	2.4	1.9	0.4	0.5	0.1	0.3
Total	45	1.9	2.1	0.04	0.2	1.3*	1.7	0.1*	0.3	0.04	0.2
Vitamins		**BetaTocopherol(mg)**		**GammaTocopherol(mg)**		**DeltaTocopherol(mg)**		**Vit-E(IU)**		**Vit-E, added(mg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Protein	18	0	0	0	0	0	0	3.7	5.8	0	0
High Protein	18	0.1	0.5	0.1	0.3	0	0	4.7	4.5	0.1	0.2
Control	9	0	0	0.1	0.3	0	0	5.2	4.2	0	0
Total	45	0.04	0.3	0.1	0.3	0	0	4.4	4.9	0.02	0.1
Vitamins		**Vit-K1(mcg)**		**Vit-K1D(mcg)**		**Vit-K2(mcg)**					
Group	n	Mean	SD	Mean	SD	Mean	SD				
Low Protein	18	51	141	1.0	2.9	0.3	0.6				
High Protein	18	30	44	1.1	2.0	0.7	2.6				
Control	9	31	33	4.1	4.7	1.3	1.9				
Total	45	38	93	1.6*	3.2	0.7	1.9				

Appendix B6a – Nutritional Analysis for Three Groups. Macronutrients and micronutrients are given for three groups: Low Protein, High Protein, and Control. Significant differences among groups were found for total calories, energy, total calories from protein, %calories from protein, total grams of protein, grams protein per kg total body mass per day, grams protein per kg lean mass per day, and micronutrients as indicated (* = $p<0.05$).

7. ANOVA Results for Nutrition Logs of Three Groups

ANOVA Results for Nutrition Logs for Three Groups						
		Sum of Squares	df	Mean Square	F	Sig.
Calories	Between Groups	3556004.522	2	1778002.261	4.488	0.017*
	Within Groups	16637758.72	42	396137.112		
	Total	20193763.24	44			
Energy(kj)	Between Groups	62548225.94	2	31274112.97	4.503	0.017*
	Within Groups	291720120.1	42	6945717.144		
	Total	354268346	44			
Protein(g)	Between Groups	30951.589	2	15475.794	14.416	<0.001*
	Within Groups	45087.389	42	1073.509		
	Total	76038.978	44			
Protein(g/kg TOTAL)	Between Groups	6.764	2	3.382	20.381	<0.001*
	Within Groups	6.97	42	0.166		
	Total	13.734	44			
Protein(g/kg LEAN)	Between Groups	5.133	2	2.566	7.32	0.002*
	Within Groups	14.726	42	0.351		
	Total	19.859	44			
Calories/Protein	Between Groups	517839.367	2	258919.683	14.189	<0.001*
	Within Groups	766431.611	42	18248.372		
	Total	1284270.978	44			
%Calories/Protein	Between Groups	163.333	2	81.667	3.347	0.045*
	Within Groups	1024.667	42	24.397		
	Total	1188	44			
Carbohydrates(g)	Between Groups	32221.856	2	16110.928	2.528	0.092
	Within Groups	267663.389	42	6372.938		
	Total	299885.244	44			
Starch(g)	Between Groups	1873.2	2	936.6	2.381	0.105
	Within Groups	16520	42	393.333		
	Total	18393.2	44			
Sugars(g)	Between Groups	4053.5	2	2026.75	1.379	0.263
	Within Groups	61744.944	42	1470.118		
	Total	65798.444	44			
Glucose(g)	Between Groups	64.911	2	32.456	1.137	0.33
	Within Groups	1199	42	28.548		
	Total	1263.911	44			
Fructose(g)	Between Groups	55.633	2	27.817	1.109	0.339
	Within Groups	1053.611	42	25.086		
	Total	1109.244	44			
Galactose(g)	Between Groups	0	2	0	.	.
	Within Groups	0	42	0		
	Total	0	44			
Sucrose(g)	Between Groups	13.8	2	6.9	0.793	0.459
	Within Groups	365.444	42	8.701		
	Total	379.244	44			
Lactose(g)	Between Groups	4.556	2	2.278	0.781	0.465
	Within Groups	122.556	42	2.918		
	Total	127.111	44			
Maltose(g)	Between Groups	1.778	2	0.889	1.6	0.214
	Within Groups	23.333	42	0.556		
	Total	25.111	44			
Fiber(g)	Between Groups	295.2	2	147.6	1.322	0.278
	Within Groups	4689.778	42	111.661		
	Total	4984.978	44			
Estimated Net Carb(g)	Between Groups	36752.8	2	18376.4	3.141	0.054
	Within Groups	245737.111	42	5850.884		
	Total	282489.911	44			
Calories/Carbohydrate	Between Groups	520862.667	2	260431.333	2.804	0.072
	Within Groups	3900755.111	42	92875.122		
	Total	4421617.778	44			
%Calories/Carbohydrate	Between Groups	15.633	2	7.817	0.128	0.88
	Within Groups	2566.944	42	61.118		
	Total	2582.578	44			
Fat(g)	Between Groups	3506.3	2	1753.15	1.693	0.196
	Within Groups	43485.611	42	1035.372		
	Total	46991.911	44			
Saturated Fat(g)	Between Groups	423.244	2	211.622	2.284	0.114
	Within Groups	3891.556	42	92.656		
	Total	4314.8	44			

ANOVA Results for Nutrition Logs for Three Groups cont'd.						
		Sum of Squares	df	Mean Square	F	Sig.
Trans-MonounsaturatedFat(g)	Between Groups	0.089	2	0.044	2.1	0.135
	Within Groups	0.889	42	0.021		
	Total	0.978	44			
Trans-PolyunsaturatedFat(g)	Between Groups	0	2	0	.	.
	Within Groups	0	42	0		
	Total	0	44			
Monounsaturated Fat(g)	Between Groups	378.8	2	189.4	1.596	0.215
	Within Groups	4983.778	42	118.661		
	Total	5362.578	44			
Polyunsaturated Fat(g)	Between Groups	104.3	2	52.15	2.046	0.142
	Within Groups	1070.5	42	25.488		
	Total	1174.8	44			
Omega3(g)	Between Groups	0.389	2	0.194	0.812	0.451
	Within Groups	10.056	42	0.239		
	Total	10.444	44			
Omega6(g)	Between Groups	36.922	2	18.461	3.123	0.054
	Within Groups	248.278	42	5.911		
	Total	285.2	44			
Calories/Fat	Between Groups	289486.7	2	144743.35	1.628	0.208
	Within Groups	3734501.611	42	88916.705		
	Total	4023988.311	44			
%Calories/Fat	Between Groups	89.444	2	44.722	1.167	0.321
	Within Groups	1609.667	42	38.325		
	Total	1699.111	44			
Cholesterol(mg)	Between Groups	862468.8	2	431234.4	8.4	0.001*
	Within Groups	2156124.444	42	51336.296		
	Total	3018593.244	44			
Alcohol(g)	Between Groups	8.2	2	4.1	0.512	0.603
	Within Groups	336.111	42	8.003		
	Total	344.311	44			
Calories/Alcohol	Between Groups	384.133	2	192.067	0.491	0.616
	Within Groups	16436.444	42	391.344		
	Total	16820.578	44			
%Calories/Alcohol	Between Groups	0.667	2	0.333	0.516	0.6
	Within Groups	27.111	42	0.646		
	Total	27.778	44			
Ash(g)	Between Groups	182.7	2	91.35	5.337	0.009*
	Within Groups	718.944	42	17.118		
	Total	901.644	44			
Caffeine(mg)	Between Groups	662.033	2	331.017	0.159	0.854
	Within Groups	87501.611	42	2083.372		
	Total	88163.644	44			
Theobromine(mg)	Between Groups	3445.644	2	1722.822	3.575	0.037*
	Within Groups	20239.556	42	481.894		
	Total	23685.2	44			
Phytosterol(mg)	Between Groups	348.2	2	174.1	1.346	0.271
	Within Groups	5431	42	129.31		
	Total	5779.2	44			
Stigmasterol(mg)	Between Groups	0.167	2	0.083	0.364	0.697
	Within Groups	9.611	42	0.229		
	Total	9.778	44			
Campesterol(mg)	Between Groups	0.467	2	0.233	0.323	0.726
	Within Groups	30.333	42	0.722		
	Total	30.8	44			
Beta-sitosterol(mg)	Between Groups	322.7	2	161.35	0.959	0.392
	Within Groups	7066.278	42	168.245		
	Total	7388.978	44			
Vit-A(mcg RAE)	Between Groups	142537.356	2	71268.678	1.499	0.235
	Within Groups	1996781.889	42	47542.426		
	Total	2139319.244	44			
Retinol(mcg)	Between Groups	128480.2	2	64240.1	5.335	0.009*
	Within Groups	505751	42	12041.69		
	Total	634231.2	44			
BetaCarotene(mcg)	Between Groups	4693578.181	2	2346789.09	0.632	0.537
	Within Groups	152204520.1	41	3712305.37		
	Total	156898098.3	43			

ANOVA Results for Nutrition Logs for Three Groups cont'd.						
		Sum of Squares	df	Mean Square	F	Sig.
AlphaCarotene(mcg)	Between Groups	150452.967	2	75226.483	0.326	0.724
	Within Groups	9698331.833	42	230912.663		
	Total	9848784.8	44			
BetaCryptoxanthin(mcg)	Between Groups	1506.089	2	753.044	0.42	0.66
	Within Groups	75247.111	42	1791.598		
	Total	76753.2	44			
Lycopene(mcg)	Between Groups	3698809.533	2	1849404.767	0.613	0.547
	Within Groups	126746578.8	42	3017775.685		
	Total	130445388.3	44			
Lutein+Zeaxanthin(mcg)	Between Groups	104994.689	2	52497.344	0.032	0.968
	Within Groups	68506814.56	42	1631114.632		
	Total	68611809.24	44			
Vit-A IU	Between Groups	19731737.2	2	9865868.6	0.73	0.488
	Within Groups	567927706.4	42	13522088.25		
	Total	587659443.6	44			
Vit-B1(mg)	Between Groups	0.967	2	0.483	1.153	0.326
	Within Groups	17.611	42	0.419		
	Total	18.578	44			
Vit-B2(mg)	Between Groups	2.333	2	1.167	0.48	0.622
	Within Groups	102.111	42	2.431		
	Total	104.444	44			
Vit-B3(mg)	Between Groups	60.644	2	30.322	0.178	0.838
	Within Groups	7158.556	42	170.442		
	Total	7219.2	44			
Vit-B5(mg)	Between Groups	9.911	2	4.956	0.358	0.701
	Within Groups	580.667	42	13.825		
	Total	590.578	44			
Vit-B6(mg)	Between Groups	0.911	2	0.456	0.21	0.811
	Within Groups	91	42	2.167		
	Total	91.911	44			
Choline(mg)	Between Groups	368570.033	2	184285.017	4.78	0.013*
	Within Groups	1619147.167	42	38551.123		
	Total	1987717.2	44			
Folate(mcg)	Between Groups	40200.133	2	20100.067	1.699	0.195
	Within Groups	496953.111	42	11832.217		
	Total	537153.244	44			
FoodFolate(mcg)	Between Groups	20560.3	2	10280.15	1.571	0.22
	Within Groups	274826.278	42	6543.483		
	Total	295386.578	44			
FolicAcid(mcg)	Between Groups	2926.533	2	1463.267	0.568	0.571
	Within Groups	108234.667	42	2577.016		
	Total	111161.2	44			
Dietary Folate Equivalents (mcgDFE)	Between Groups	61645.533	2	30822.767	2.011	0.147
	Within Groups	643734.111	42	15327.003		
	Total	705379.644	44			
Vit-B12(mcg)	Between Groups	8.889	2	4.444	0.202	0.818
	Within Groups	926.222	42	22.053		
	Total	935.111	44			
Vit-B12, added(mcg)	Between Groups	7.311	2	3.656	0.39	0.68
	Within Groups	393.889	42	9.378		
	Total	401.2	44			
Vit-H(mcg)	Between Groups	4.089	2	2.044	0.173	0.841
	Within Groups	495.111	42	11.788		
	Total	499.2	44			
Vit-C(mg)	Between Groups	4777.144	2	2388.572	0.441	0.646
	Within Groups	227272.5	42	5411.25		
	Total	232049.644	44			
Vit-D(IU)	Between Groups	99097.189	2	49548.594	5.727	0.006*
	Within Groups	363354.722	42	8651.303		
	Total	462451.911	44			
Vit-D2(mcg)	Between Groups	0	2	0	.	.
	Within Groups	0	42	0		
	Total	0	44			
Vit-D3(mcg)	Between Groups	29.811	2	14.906	4.366	0.019*
	Within Groups	143.389	42	3.414		
	Total	173.2	44			

ANOVA Results for Nutrition Logs for Three Groups cont'd.						
		Sum of Squares	df	Mean Square	F	Sig.
Vit-D2+D3(mcg)	Between Groups	43.867	2	21.933	6.437	0.004*
	Within Groups	143.111	42	3.407		
	Total	186.978	44			
AlphaTocopherol(mg)	Between Groups	13.589	2	6.794	1.659	0.203
	Within Groups	172.056	42	4.097		
	Total	185.644	44			
BetaTocopherol(mg)	Between Groups	0.078	2	0.039	0.891	0.418
	Within Groups	1.833	42	0.044		
	Total	1.911	44			
GammaTocopherol(mg)	Between Groups	21.856	2	10.928	4.639	0.015*
	Within Groups	98.944	42	2.356		
	Total	120.8	44			
DeltaTocopherol(mg)	Between Groups	1.2	2	0.6	6.3	0.004*
	Within Groups	4	42	0.095		
	Total	5.2	44			
AlphaTocotrienol(mg)	Between Groups	0.078	2	0.039	0.891	0.418
	Within Groups	1.833	42	0.044		
	Total	1.911	44			
BetaTocotrienol(mg)	Between Groups	0.133	2	0.067	0.741	0.483
	Within Groups	3.778	42	0.09		
	Total	3.911	44			
GammaTocotrienol(mg)	Between Groups	0.133	2	0.067	1.05	0.359
	Within Groups	2.667	42	0.063		
	Total	2.8	44			
DeltaTocotrienol(mg)	Between Groups	0	2	0	.	.
	Within Groups	0	42	0		
	Total	0	44			
Vit-E(IU)	Between Groups	15.633	2	7.817	0.311	0.734
	Within Groups	1055.167	42	25.123		
	Total	1070.8	44			
Vit-E, added(mg)	Between Groups	0.033	2	0.017	0.741	0.483
	Within Groups	0.944	42	0.022		
	Total	0.978	44			
Vit-K1(mcg)	Between Groups	4825.411	2	2412.706	0.268	0.766
	Within Groups	378625.833	42	9014.901		
	Total	383451.244	44			
Vit-K1D(mcg)	Between Groups	68.478	2	34.239	3.67	0.034*
	Within Groups	391.833	42	9.329		
	Total	460.311	44			
Vit-K2(mcg)	Between Groups	6.778	2	3.389	0.941	0.398
	Within Groups	151.222	42	3.601		
	Total	158	44			
Betaine(mg)	Between Groups	8137.889	2	4068.944	2.193	0.124
	Within Groups	77910.556	42	1855.013		
	Total	86048.444	44			
Calcium(mg)	Between Groups	489298.522	2	244649.261	2.653	0.082
	Within Groups	3872413.389	42	92200.319		
	Total	4361711.911	44			
Chloride(mg)	Between Groups	58.8	2	29.4	0.741	0.483
	Within Groups	1666	42	39.667		
	Total	1724.8	44			
Magnesium(mg)	Between Groups	64096.2	2	32048.1	4.155	0.023*
	Within Groups	323957	42	7713.262		
	Total	388053.2	44			
Phosphate(mg)	Between Groups	2537589.422	2	1268794.711	8.046	0.001*
	Within Groups	6623061.556	42	157691.942		
	Total	9160650.978	44			
Potassium(mg)	Between Groups	3614030.867	2	1807015.433	3.57	0.037*
	Within Groups	21256249.44	42	506101.177		
	Total	24870280.31	44			
Sodium(mg)	Between Groups	11334115.91	2	5667057.956	3.387	0.043*
	Within Groups	70282678.89	42	1673397.116		
	Total	81616794.8	44			
Chromium(mcg)	Between Groups	0.7	2	0.35	0.191	0.827
	Within Groups	76.944	42	1.832		
	Total	77.644	44			

ANOVA Results for Nutrition Logs for Three Groups cont'd.						
		Sum of Squares	df	Mean Square	F	Sig.
Copper(mg)	Between Groups	1.556	2	0.778	2.262	0.117
	Within Groups	14.444	42	0.344		
	Total	16	44			
Fluoride(mcg)	Between Groups	68967.5	2	34483.75	1.034	0.364
	Within Groups	1400640.278	42	33348.578		
	Total	1469607.778	44			
Iodine(mcg)	Between Groups	1.556	2	0.778	0.162	0.851
	Within Groups	201.556	42	4.799		
	Total	203.111	44			
Iron(mg)	Between Groups	35.278	2	17.639	0.655	0.525
	Within Groups	1130.722	42	26.922		
	Total	1166	44			
Manganese(mg)	Between Groups	4.467	2	2.233	1.823	0.174
	Within Groups	51.444	42	1.225		
	Total	55.911	44			
Molybdenum(mcg)	Between Groups	0.2	2	0.1	0.105	0.901
	Within Groups	40.111	42	0.955		
	Total	40.311	44			
Selenium(mcg)	Between Groups	23994.8	2	11997.4	7.585	0.002*
	Within Groups	66434	42	1581.762		
	Total	90428.8	44			
Zinc(mg)	Between Groups	50.078	2	25.039	0.931	0.402
	Within Groups	1129.167	42	26.885		
	Total	1179.244	44			
Histidine(g)	Between Groups	8.644	2	4.322	5.985	0.005*
	Within Groups	30.333	42	0.722		
	Total	38.978	44			
Isoleucine(g)	Between Groups	17.5	2	8.75	5.49	0.008*
	Within Groups	66.944	42	1.594		
	Total	84.444	44			
Leucine(g)	Between Groups	51.5	2	25.75	5.625	0.007*
	Within Groups	192.278	42	4.578		
	Total	243.778	44			
Lysine(g)	Between Groups	49	2	24.5	5.979	0.005*
	Within Groups	172.111	42	4.098		
	Total	221.111	44			
Methionine(g)	Between Groups	5.367	2	2.683	5.162	0.01*
	Within Groups	21.833	42	0.52		
	Total	27.2	44			
Phenylalanine(g)	Between Groups	14.333	2	7.167	5.364	0.008*
	Within Groups	56.111	42	1.336		
	Total	70.444	44			
Threonine(g)	Between Groups	12.889	2	6.444	4.545	0.016*
	Within Groups	59.556	42	1.418		
	Total	72.444	44			
Tryptophan(g)	Between Groups	1.244	2	0.622	3.564	0.037*
	Within Groups	7.333	42	0.175		
	Total	8.578	44			
Valine(g)	Between Groups	21.8	2	10.9	5.715	0.006*
	Within Groups	80.111	42	1.907		
	Total	101.911	44			
Alanine(g)	Between Groups	25.2	2	12.6	6.615	0.003*
	Within Groups	80	42	1.905		
	Total	105.2	44			
Arginine(g)	Between Groups	27.311	2	13.656	5.36	0.008*
	Within Groups	107	42	2.548		
	Total	134.311	44			
Aspartic Acid(g)	Between Groups	65.411	2	32.706	5.193	0.01*
	Within Groups	264.5	42	6.298		
	Total	329.911	44			
Cystine(g)	Between Groups	2.7	2	1.35	4.883	0.012*
	Within Groups	11.611	42	0.276		
	Total	14.311	44			
Glutamic Acid(g)	Between Groups	245.467	2	122.733	6.531	0.003*
	Within Groups	789.333	42	18.794		
	Total	1034.8	44			

ANOVA Results for Nutrition Logs for Three Groups cont'd.						
		Sum of Squares	df	Mean Square	F	Sig.
Glycine(g)	Between Groups	15.422	2	7.711	7.007	0.002*
	Within Groups	46.222	42	1.101		
	Total	61.644	44			
Hydroxyproline(g)	Between Groups	0.089	2	0.044	2.1	0.135
	Within Groups	0.889	42	0.021		
	Total	0.978	44			
Proline(g)	Between Groups	22.478	2	11.239	5.32	0.009*
	Within Groups	88.722	42	2.112		
	Total	111.2	44			
Serine(g)	Between Groups	15.944	2	7.972	4.29	0.02*
	Within Groups	78.056	42	1.858		
	Total	94	44			
Tyrosine(g)	Between Groups	11.5	2	5.75	6.273	0.004*
	Within Groups	38.5	42	0.917		
	Total	50	44			

Appendix B7 – ANOVA Results for Nutrition Logs of Three Groups. Macronutrients and micronutrients were analyzed with a one-way ANOVA for three groups: Low Protein, High Protein, and Control. Significant differences among groups were found for total calories, energy, total calories from protein, %calories from protein, total grams of protein, grams protein per kg total body mass per day, grams protein per kg lean mass per day, and micronutrients as indicated (* = $p<0.05$).

8. Tukey Post-Hoc Test for Nutrition Logs of Three Groups

Tukey HSD Post-Hoc Test for Nutrition Logs for Three Groups					
Dependent Variable	(I) GROUP	(J) GROUP	Mean Difference (I-J)	SD	Sig.
Calories	LO	HI	-623.83333	209.79808	0.013
Energy(kj)	LO	HI	-2615.72222	878.49095	0.013
Protein(g)	LO	HI	-57.83333	10.92148	<0.001
	HI	CON	39.77778	13.37603	0.013
Protein(g/kg TOTAL)	LO	HI	-0.85224	0.13579	<0.001
	HI	CON	0.60382	0.1663	0.002
Protein (g/kg LEAN)	LO	HI	-0.73592	0.19738	0.002
Calories/Protein	LO	HI	-236.72222	45.02884	<0.001
	HI	CON	161.66667	55.14885	0.015
%Calories/Protein	HI	CON	5	2.01647	0.045
Estimated Net Carb(g)	LO	HI	-63.55556	25.49702	0.043
Omega6(g)	LO	CON	-2.44444	0.99259	0.046
Cholesterol(mg)	LO	HI	-188.22222	75.52505	0.043
		CON	-368.88889	92.49892	0.001
Ash(g)	LO	CON	-5.5	1.68907	0.006
Retinol(mcg)	LO	CON	-137.61111	44.79898	0.01
	HI	CON	-128.83333	44.79898	0.017
Choline(mg)	LO	CON	-247.5	80.15727	0.01
Vit-D(IU)	LO	CON	-127.55556	37.97215	0.005
	HI	CON	-96.72222	37.97215	0.038
Vit-D3(mcg)	LO	CON	-2.22222	0.75432	0.014
Vit-D2+D3(mcg)	LO	CON	-2.66667	0.75359	0.003
	HI	CON	-2.11111	0.75359	0.021
GammaTocopherol(mg)	LO	CON	-1.88889	0.62661	0.012
DeltaTocopherol(mg)	LO	CON	-0.44444	0.12599	0.003
	HI	CON	-0.33333	0.12599	0.03
Vit-K1D(mcg)	LO	CON	-3.11111	1.24695	0.043
	HI	CON	-3.05556	1.24695	0.048
Magnesium(mg)	LO	CON	-99.83333	35.85448	0.021
Phosphate(mg)	LO	CON	-650.11111	162.11721	0.001
	HI	CON	-420.77778	162.11721	0.034
Potassium(mg)	LO	CON	-764.05556	290.43105	0.031
Selenium(mcg)	LO	CON	-61.66667	16.23659	0.001
Histidine(g)	LO	HI	-0.77778	0.28328	0.024
		CON	-1.05556	0.34694	0.011
	HI	LO	0.77778	0.28328	0.024
Isoleucine(g)	LO	CON	-1.66667	0.51541	0.007
Leucine(g)	LO	CON	-2.83333	0.8735	0.006
Lysine(g)	LO	CON	-2.72222	0.82643	0.006
Methionine(g)	LO	HI	-0.61111	0.24033	0.038
		CON	-0.83333	0.29435	0.019
Phenylalanine(g)	LO	CON	-1.5	0.47187	0.008
Threonine(g)	LO	CON	-1.44444	0.48614	0.013
Tryptophan(g)	LO	CON	-0.44444	0.17059	0.033
Valine(g)	LO	CON	-1.83333	0.56383	0.006
Alanine(g)	LO	CON	-2	0.56344	0.003
Arginine(g)	LO	CON	-2.05556	0.65162	0.008
Aspartic Acid(g)	LO	CON	-3.22222	1.0245	0.008
Cystine(g)	LO	CON	-0.66667	0.21465	0.009
Glutamic Acid(g)	LO	CON	-6.33333	1.76982	0.003
Glycine(g)	LO	HI	-1	0.34969	0.018
		CON	-1.44444	0.42828	0.004
Proline(g)	LO	CON	-1.88889	0.59336	0.008
Serine(g)	LO	CON	-1.61111	0.55655	0.016

Appendix B8 – Tukey Post-Hoc Test for Nutrition Logs of Three Groups. Macronutrients and micronutrients were analyzed with a one-way ANOVA and Tukey HSD for three groups: LO, HI, and CON. Table shows all significant relationships ($p<0.05$).

9. Nutritional Analysis for Five Groups

Calorie Information		Calories		Energy(kj)		Calories/Protein		%Calories/Protein		Calories/Carbohydrate	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	1750	483	7322	2021	457	80	27	6	681	251
2 (LO/IPE)	70	2313	579	9658	2446	462	104	21	2	1084	268
3 (HI/DPE)	71	2592	628	10840	2626	685	175	27	6	1101	286
4 (HI/IPE)	70	2718	325	11372	1358	707	59	26	3	1144	278
5 (CON)	71	2429	908	10165	3798	534	210	22	4	1017	318
Total	353	2360*	677	9871*	2838	569*	171	25*	5	1005*	317
Calorie Information		**%Calories/Carbohydrate**		**Calories/Fat**		**%Calories/Fat**		**Calories/Alcohol**		**%Calories/Alcohol**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	38	10	602	246	34	8	10.9	26.3	0.4	1.0
2 (LO/IPE)	70	47	6	767	258	33	5	0.0	0.0	0.0	0.0
3 (HI/DPE)	71	42	4	806	261	31	4	0.0	0.0	0.0	0.0
4 (HI/IPE)	70	42	6	846	150	31	5	16.0	33.9	0.7	1.4
5 (CON)	71	44	9	878	482	35	8	0.0	0.0	0.0	0.0
Total	353	43	8	780	302	33	6	5.4	19.6	0.2	0.8

Proteins		Protein(g)		Protein(g/kg TOTAL)		Protein(g/kg LEAN)		Isoleucine(g)		Leucine(g)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	112	19	1.2	0.3	1.9	0.5	0.3	0.5	0.8	0.7
2 (LO/IPE)	70	113	24	1.4	0.3	2.0	0.5	0.1	0.3	0.4	0.5
3 (HI/DPE)	71	169	42	2.2	0.4	2.6	0.6	1.0	1.1	1.8	1.9
4 (HI/IPE)	70	171	16	2.1	0.3	2.7	0.4	1.1	0.9	2.4	1.5
5 (CON)	71	130	51	1.6	0.7	2.1	0.9	1.9	2.4	3.4	4.2
Total	353	139*	42	1.7*	0.6	2.2*	0.7	0.9*	1.4	1.8*	2.4
Proteins		**Lysine(g)**		**Methionine(g)**		**Phenylalanine(g)**		**Threonine(g)**		**Tryptophan(g)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	0.7	0.7	0.1	0.3	0.3	0.5	0.7	1.0	0	0
2 (LO/IPE)	70	0.1	0.3	0	0	0.2	0.4	0	0	0	0
3 (HI/DPE)	71	1.7	1.9	0.6	0.7	0.9	0.9	0.9	0.9	0.2	0.4
4 (HI/IPE)	70	2.2	1.4	0.8	0.7	1.2	1.1	1.1	0.9	0.2	0.4
5 (CON)	71	3.1	3.9	0.9	1.3	1.8	2.1	1.8	2.1	0.4	0.7
Total	353	1.6*	2.2	0.5*	0.8	0.9*	1.3	0.9*	1.3	0.2	0.4
Proteins		**Valine(g)**		**Alanine(g)**		**Arginine(g)**		**Aspartic Acid(g)**		**Cystine(g)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	0.6	0.5	0.4	0.5	0.8	0.7	0.8	0.7	0	0
2 (LO/IPE)	70	0.2	0.4	0.2	0.4	0.2	0.4	0.6	0.5	0	0
3 (HI/DPE)	71	1.2	1.2	1.2	1.2	1.4	1.5	1.9	2.0	0.3	0.5
4 (HI/IPE)	70	1.6	1.0	1.4	1.0	1.8	1.1	2.7	1.3	0.2	0.4
5 (CON)	71	2.2	2.6	2.3	2.6	2.6	3.0	3.9	5.1	0.7	1.0
Total	353	1.2*	1.5	1.1*	1.5	1.4*	1.7	2.0*	2.7	0.2	0.6
Proteins		**Glutamic Acid(g)**		**Glycine(g)**		**Hydroxyproline(g)**		**Proline(g)**		**Serine(g)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	1.7	1.4	0.3	0.5	0	0	0.6	0.5	0.6	0.5
2 (LO/IPE)	70	1.4	1.3	0.1	0.3	0	0	0.3	0.5	0.2	0.4
3 (HI/DPE)	71	3.8	3.9	1.1	1.3	0	0	1.1	1.3	0.9	0.9
4 (HI/IPE)	70	4.9	2.8	1.3	0.7	0	0	1.7	1.1	1.3	0.9
5 (CON)	71	7.9	8.4	1.7	1.8	0.1	0.3	2.3	2.7	2.0	2.7
Total	353	3.9*	4.8	0.9*	1.2	0.02	0.1	1.2*	1.6	1.0	1.5
Proteins		**Tyrosine(g)**		**Histidine(g)**							
Group	n	Mean	SD	Mean	SD						
1 (LO/DPE)	71	0.2	0.4	0.1	0.3						
2 (LO/IPE)	70	0	0	0	0						
3 (HI/DPE)	71	0.7	0.9	0.7	0.9						
4 (HI/IPE)	70	1.0	0.7	1.0	0.7						
5 (CON)	71	1.4	1.8	1.1	1.5						
Total	353	0.7*	1.1	0.6*	0.9						

Carbohydrates		Carbohydrate(g)		Starch(g)		Sugars(g)		Glucose(g)		Fructose(g)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	188	63	5	4	44	37	1.9	3.1	1.8	2.8
2 (LO/IPE)	70	290	73	11	8	73	40	5.3	9.3	5.1	6.3
3 (HI/DPE)	71	293	80	14	18	64	26	2.1	2.1	2.2	2.4
4 (HI/IPE)	70	304	71	18	25	91	44	4.9	3.9	5.9	5.0
5 (CON)	71	268	83	25	31	78	34	6.6	4.9	6.4	6.3
Total	353	268*	83	14	20	70	39	4.2	5.4	4.3	5.0
Carbohydrates		**Galactose(g)**		**Sucrose(g)**		**Lactose(g)**		**Maltose(g)**		**Fiber(g)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	0	0	1.1	1.9	0.1	0.3	0.1	0.3	23	10
2 (LO/IPE)	70	0	0	4.3	3.6	0	0	0.3	0.5	27	14
3 (HI/DPE)	71	0	0	2.1	1.5	0	0	0.7	0.9	24	13
4 (HI/IPE)	70	0	0	4.7	3.4	1.4	3.4	0.7	1.1	30	9
5 (CON)	71	0	0	4.2	2.4	0.7	1.7	0.4	0.7	20.2	5.8
Total	353	0	0	3.3*	2.9	0.4	1.7	0.4	0.8	25.0	10.6

Fats		Fat(g)		Saturated Fat(g)		TransFat(g)		ans-Monounsaturated Fat		rans-Polyunsaturated Fat(	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	65	26	20	9	0.8	0.8	0	0	0	0
2 (LO/IPE)	70	82	27	21	7	0.2	0.4	0	0	0	0
3 (HI/DPE)	71	87	28	24	8	0.3	0.5	0	0	0	0
4 (HI/IPE)	70	90	17	26	8	0.7	0.7	0	0	0	0
5 (CON)	71	95	53	28	16	0.7	1.0	0.1	0.3	0	0
Total	353	84	33	24	10	0.5	0.7	0	0.1	0	0
Fats		**Monounsaturated Fat(g)**		**Polyunsaturated Fat(g)**		**Omega3(g)**		**Omega6(g)**			
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
1 (LO/DPE)	71	7	4	4	3	0	0	0.7	1.1		
2 (LO/IPE)	70	10	9	4	2	0	0	0.9	1.4		
3 (HI/DPE)	71	8	5	5	4	0.3	1.0	1.2	1.3		
4 (HI/IPE)	70	12	6	7	3	0	0	1.3	1.0		
5 (CON)	71	17	21	8	10	0.2	0.4	3.2	5.0		
Total	353	11	11	6	5	0.1	0.5	1.5	2.5		

Sterols		Cholesterol(mg)		Phytosterol(mg)		Stigmasterol(mg)		Campesterol(mg)		Beta-sitosterol(mg)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	219	72	4.0	5.5	0.2	0.4	0.3	0.7	6.7	14.3
2 (LO/IPE)	70	172	58	6.3	9.9	0.2	0.4	0.7	1.1	10.2	18.0
3 (HI/DPE)	71	368	110	5.2	6.5	0	0	0.2	0.7	3.6	10.7
4 (HI/IPE)	70	399	237	15.6	14.7	0.3	0.7	0.6	1.1	8.3	14.7
5 (CON)	71	564	437	11.6	15.1	0.3	0.5	0.2	0.4	1.1	1.7
Total	353	345*	262	8.5	11.5	0.2	0.5	0.4	0.8	6.0	13.0

Other		Alcohol(g)		Ash(g)		Caffeine(mg)		Theobromine(mg)		Choline(mg)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	1.4	3.6	1.9	1.5	4	13	2	6	35	27
2 (LO/IPE)	70	0	0	3.6	2.5	35	63	2	3	25	23
3 (HI/DPE)	71	0	0	4.1	3.3	4	11	0	1	70	62
4 (HI/IPE)	70	2.3	4.9	5.7	4.1	28	75	6	12	174	161
5 (CON)	71	0	0	8.2	7.1	9	11	25	48	278	406
Total	353	0.8	2.8	4.7*	4.5	16	45	7	23	116*	213
Other		**Betaine(mg)**									
Group	n	Mean	SD								
1 (LO/DPE)	71	2	3								
2 (LO/IPE)	70	3	3								
3 (HI/DPE)	71	46	92								
4 (HI/IPE)	70	17	27								
5 (CON)	71	8	9								
Total	353	15	44								

Vitamins		Vit-A(mcg RAE)		Retinol(mcg)		BetaCarotene(mcg)		AlphaCarotene(mcg)		BetaCryptoxanthin(mcg)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	104	152	23	25	926	1559	191	511	11	15
2 (LO/IPE)	70	200	182	82	79	1180	1932	163	291	37	54
3 (HI/DPE)	71	179	196	26	40	1651	2054	365	743	16	32
4 (HI/IPE)	70	297	273	97	96	1864	2460	200	369	14	24
5 (CON)	71	298	259	190	202	1169	1684	147	403	30	65
Total	353	216	221	84*	120	1368	1910	213	473	21	42
Vitamins		**Lycopene(mcg)**		**Lutein+Zeaxanthin(mcg)**		**Vit-A IU**		**Vit-B1(mg)**		**Vit-B2(mg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	160	290	344	516	2851	2918	0.3	0.5	0.2	0.4
2 (LO/IPE)	70	440	872	899	2273	4012	3944	0.6	0.5	1.4	3.2
3 (HI/DPE)	71	1073	3219	638	1327	4701	3206	0.4	0.5	0.4	0.5
4 (HI/IPE)	70	752	2045	616	657	5083	4422	1.0	0.7	1.0	0.5
5 (CON)	71	394	594	745	876	4435	4030	0.8	0.8	1.3	0.7
Total	353	564	1722	648	1249	4216	3655	0.6	0.6	0.9	1.5
Vitamins		**Vit-B3(mg)**		**Vit-B5(mg)**		**Vit-B6(mg)**		**Folate(mcg)**		**FoodFolate(mcg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	6.2	4.7	0.7	0.7	0.1	0.3	93	69	50	54
2 (LO/IPE)	70	15.9	23.5	3.3	7.4	1.8	2.7	143	117	63	98
3 (HI/DPE)	71	12.6	11.0	1.3	0.9	0.7	0.9	147	100	69	74
4 (HI/IPE)	70	14.0	7.8	2.4	1.6	1.0	0.7	201	106	102	64
5 (CON)	71	13.7	9.2	3.1	3.0	1.2	1.0	188	139	113	107
Total	353	12.5	12.8	2.2	3.7	1.0	1.4	154	110	79	82
Vitamins		**FolicAcid(mcg)**		**ıry Folate Equivalents(mcg**		**Vit-B12(mcg)**		**Vit-B12, added(mcg)**		**Vit-H(mcg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	7	9	46	36	0.4	0.5	0	0	0	0
2 (LO/IPE)	70	50	61	147	111	4.0	8.3	2.1	4.5	2.2	6.7
3 (HI/DPE)	71	35	58	114	109	0.9	0.8	0.1	0.3	0	0
4 (HI/IPE)	70	34	56	158	146	3.6	5.2	1.8	5.0	0.9	2.7
5 (CON)	71	51	46	197	164	3.3	2.8	0	0	0.9	2.7
Total	353	36	50	132	127	2.4	4.6	0.8	3.0	0.8	3.4
Vitamins		**Vit-C(mg)**		**Vit-D(IU)**		**Vit-D2(mcg)**		**Vit-D3(mcg)**		**Vit-D2+D3(mcg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	82	142	21	38	0	0	0	0	0	0
2 (LO/IPE)	70	60	58	49	77	0	0	0.2	0.4	0.4	0.7
3 (HI/DPE)	71	42	38	44	44	0	0	0.1	0.3	0.2	0.4
4 (HI/IPE)	70	56	45	88	78	0	0	1.3	1.5	1.3	1.5
5 (CON)	71	51	32	163	169	0	0	2.3	3.8	2.9	3.8
Total	353	58	73	73	103	0	0	0.8*	2.0	1.0*	2.1
Vitamins		**AlphaTocopherol(mg)**		**BetaTocopherol(mg)**		**GammaTocopherol(mg)**		**DeltaTocopherol(mg)**		**AlphaTocopherol(mg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	1.0	1.9	0	0	0.2	0.4	0	0	0	0
2 (LO/IPE)	70	2.1	2.8	0	0	0.9	0.9	0	0	0	0
3 (HI/DPE)	71	1.1	0.9	0.1	0.3	1.2	1.9	0.1	0.3	0.1	0.3
4 (HI/IPE)	70	2.3	1.3	0	0	1.6	1.9	0.1	0.3	0	0
5 (CON)	71	3.0	2.4	0.1	0.3	2.4	1.9	0.4	0.5	0.1	0.3
Total	353	1.9	2.1	0	0.2	1.3	1.7	0.1*	0.3	0	0.2
Vitamins		**BetaTocopherol(mg)**		**GammaTocopherol(mg)**		**DeltaTocopherol(mg)**		**Vit-E(IU)**		**Vit-E, added(mg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	0	0	0	0	0	0	1.9	2.8	0	0
2 (LO/IPE)	70	0	0	0	0	0	0	5.6	7.5	0	0
3 (HI/DPE)	71	0.2	0.7	0.2	0.4	0	0	4.7	4.4	0	0
4 (HI/IPE)	70	0	0	0	0	0	0	4.7	4.8	0.1	0.3
5 (CON)	71	0	0	0.1	0.3	0	0	5.2	4.2	0	0
Total	353	0	0.3	0.1	0.3	0	0	4.4	4.9	0	0.1
Vitamins		**Vit-K1(mcg)**		**Vit-K1D(mcg)**		**Vit-K2(mcg)**					
Group	n	Mean	SD	Mean	SD	Mean	SD				
1 (LO/DPE)	71	28	40	0.2	0.4	0.4	0.7				
2 (LO/IPE)	70	75	198	1.8	4.1	0.1	0.3				
3 (HI/DPE)	71	32	55	1.0	2.3	0.2	0.7				
4 (HI/IPE)	70	28	33	1.1	1.8	1.2	3.7				
5 (CON)	71	31	33	4.1	4.7	1.3	1.9				
Total	353	38	93	1.6	3.2	0.7	1.9				

Minerals		Calcium(mg)		Chloride(mg)		Magnesium(mg)		Phosphate(mg)		Potassium(mg)	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	408	287	4.7	14.0	47	29	206	156	625	397
2 (LO/IPE)	70	411	332	0	0	84	75	247	158	797	583
3 (HI/DPE)	71	544	247	0	0	104	96	367	285	819	470
4 (HI/IPE)	70	593	311	0	0	134	75	544	332	1315	739
5 (CON)	71	680	364	0	0	165	133	876	754	1475	1113
Total	353	527	315	0.9	6.3	107	94	448*	456	1006	752
Minerals		**Sodium(mg)**		**Chromium(mcg)**		**Copper(mg)**		**Fluoride(mcg)**		**Iodine(mcg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	2509	891	0	0	0	0	99	260	0	0
2 (LO/IPE)	70	3293	1023	0.9	2.7	0.2	0.7	128	272	1.3	4.0
3 (HI/DPE)	71	3767	1221	0	0	0.4	0.7	4	4	0	0
4 (HI/IPE)	70	3980	1328	0.3	1.0	0.4	0.5	68	172	0.7	2.0
5 (CON)	71	4013	1822	0.3	1.0	0.6	0.7	30	33	0.8	2.0
Total	353	3512	1362	0.3	1.3	0.3	0.6	66	183	0.6	2.1
Minerals		**Iron(mg)**		**Manganese(mg)**		**Molybdenum(mcg)**		**Selenium(mcg)**		**Zinc(mg)**	
Group	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (LO/DPE)	71	8	3	0.2	0.4	0	0	10	10	4.6	8.5
2 (LO/IPE)	70	10	5	1.0	0.9	0.6	1.7	14	11	2.6	1.6
3 (HI/DPE)	71	11	7	0.7	1.3	0	0	40	47	3.9	4.3
4 (HI/IPE)	70	10	5	1.4	1.1	0.3	1.0	46	34	5.1	2.8
5 (CON)	71	10	6	1.4	1.3	0.3	1.0	74	69	6.4	6.0
Total	353	10	5	1.0	1.1	0.2	1.0	37*	45	4.5	5.2

Appendix B9 – Nutritional Analysis for Five Groups. Macronutrients and micronutrients are given for five groups: LO/DPE, LO/IPE, HI/DPE, HI/IPE, and CON. Significant differences among groups were found for total calories, energy, total calories from carbohydrate, total calories from protein, %calories from protein, total grams of protein, grams protein per kg total body mass per day, grams protein per kg lean mass per day, and micronutrients as indicated (* = $p<0.05$).

10. ANOVA Results for Nutrition Log of Five Groups

ANOVA Results for Nutrition Logs for Five Groups						
		Sum of Squares	df	Mean Square	F	Sig.
Calories	Between Groups	5056127.911	4	1264031.978	3.34	0.019*
	Within Groups	15137635.33	40	378440.883		
	Total	20193763.24	44			
Energy(kj)	Between Groups	88382330.89	4	22095582.72	3.324	0.019*
	Within Groups	265886015.1	40	6647150.378		
	Total	354268346	44			
Protein(g)	Between Groups	30986.311	4	7746.578	6.878	<0.001*
	Within Groups	45052.667	40	1126.317		
	Total	76038.978	44			
Protein(g/kg TOTAL)	Between Groups	6.93	4	1.733	10.186	<0.001*
	Within Groups	6.804	40	0.17		
	Total	13.734	44			
Protein(g/kg LEAN)	Between Groups	5.176	4	1.294	3.526	0.015*
	Within Groups	14.682	40	0.367		
	Total	19.859	44			
Calories/Protein	Between Groups	520052.978	4	130013.244	6.805	<0.001*
	Within Groups	764218	40	19105.45		
	Total	1284270.978	44			
%Calories/Protein	Between Groups	378.889	4	94.722	4.683	0.003*
	Within Groups	809.111	40	20.228		
	Total	1188	44			
Carbohydrates(g)	Between Groups	79425.467	4	19856.367	3.603	0.013*
	Within Groups	220459.778	40	5511.494		
	Total	299885.244	44			
Starch(g)	Between Groups	2121.422	4	530.356	1.304	0.285
	Within Groups	16271.778	40	406.794		
	Total	18393.2	44			
Sugars(g)	Between Groups	11316.889	4	2829.222	2.077	0.102
	Within Groups	54481.556	40	1362.039		
	Total	65798.444	44			
Glucose(g)	Between Groups	153.022	4	38.256	1.377	0.259
	Within Groups	1110.889	40	27.772		
	Total	1263.911	44			
Fructose(g)	Between Groups	166.133	4	41.533	1.762	0.156
	Within Groups	943.111	40	23.578		
	Total	1109.244	44			
Galactose(g)	Between Groups	0	4	0	.	.
	Within Groups	0	40	0		
	Total	0	44			
Sucrose(g)	Between Groups	89.911	4	22.478	3.108	0.026*
	Within Groups	289.333	40	7.233		
	Total	379.244	44			
Lactose(g)	Between Groups	14	4	3.5	1.238	0.31
	Within Groups	113.111	40	2.828		
	Total	127.111	44			
Maltose(g)	Between Groups	2	4	0.5	0.865	0.493
	Within Groups	23.111	40	0.578		
	Total	25.111	44			
Fiber(g)	Between Groups	541.422	4	135.356	1.218	0.318
	Within Groups	4443.556	40	111.089		
	Total	4984.978	44			
Estimated Net Carb(g)	Between Groups	91353.022	4	22838.256	4.779	0.003*
	Within Groups	191136.889	40	4778.422		
	Total	282489.911	44			
Calories/Carbohydrate	Between Groups	1260469.778	4	315117.444	3.987	0.008*
	Within Groups	3161148	40	79028.7		
	Total	4421617.778	44			
%Calories/Carbohydrate	Between Groups	337.911	4	84.478	1.505	0.219
	Within Groups	2244.667	40	56.117		
	Total	2582.578	44			
Fat(g)	Between Groups	4948.356	4	1237.089	1.177	0.336
	Within Groups	42043.556	40	1051.089		
	Total	46991.911	44			
Saturated Fat(g)	Between Groups	441.022	4	110.256	1.138	0.352
	Within Groups	3873.778	40	96.844		
	Total	4314.8	44			

ANOVA Results for Nutrition Logs for Five Groups cont'd.						
		Sum of Squares	df	Mean Square	F	Sig.
Trans-MonounsaturatedFat(g)	Between Groups	0.089	4	0.022	1	0.419
	Within Groups	0.889	40	0.022		
	Total	0.978	44			
Trans-PolyunsaturatedFat(g)	Between Groups	0	4	0	.	.
	Within Groups	0	40	0		
	Total	0	44			
Monounsaturated Fat(g)	Between Groups	502.578	4	125.644	1.034	0.402
	Within Groups	4860	40	121.5		
	Total	5362.578	44			
Polyunsaturated Fat(g)	Between Groups	127.022	4	31.756	1.212	0.321
	Within Groups	1047.778	40	26.194		
	Total	1174.8	44			
Omega3(g)	Between Groups	0.889	4	0.222	0.93	0.456
	Within Groups	9.556	40	0.239		
	Total	10.444	44			
Omega6(g)	Between Groups	37.2	4	9.3	1.5	0.22
	Within Groups	248	40	6.2		
	Total	285.2	44			
Calories/Fat	Between Groups	420020.978	4	105005.244	1.165	0.34
	Within Groups	3603967.333	40	90099.183		
	Total	4023988.311	44			
%Calories/Fat	Between Groups	95.333	4	23.833	0.594	0.669
	Within Groups	1603.778	40	40.094		
	Total	1699.111	44			
Cholesterol(mg)	Between Groups	876469.244	4	219117.311	4.092	0.007*
	Within Groups	2142124	40	53553.1		
	Total	3018593.244	44			
Alcohol(g)	Between Groups	42.089	4	10.522	1.393	0.254
	Within Groups	302.222	40	7.556		
	Total	344.311	44			
Calories/Alcohol	Between Groups	2069.689	4	517.422	1.403	0.25
	Within Groups	14750.889	40	368.772		
	Total	16820.578	44			
%Calories/Alcohol	Between Groups	3.556	4	0.889	1.468	0.23
	Within Groups	24.222	40	0.606		
	Total	27.778	44			
Ash(g)	Between Groups	206.089	4	51.522	2.963	0.031*
	Within Groups	695.556	40	17.389		
	Total	901.644	44			
Caffeine(mg)	Between Groups	7334.089	4	1833.522	0.907	0.469
	Within Groups	80829.556	40	2020.739		
	Total	88163.644	44			
Theobromine(mg)	Between Groups	3607.867	4	901.967	1.797	0.148
	Within Groups	20077.333	40	501.933		
	Total	23685.2	44			
Phytosterol(mg)	Between Groups	853.2	4	213.3	1.732	0.162
	Within Groups	4926	40	123.15		
	Total	5779.2	44			
Stigmasterol(mg)	Between Groups	0.667	4	0.167	0.732	0.576
	Within Groups	9.111	40	0.228		
	Total	9.778	44			
Campesterol(mg)	Between Groups	1.467	4	0.367	0.5	0.736
	Within Groups	29.333	40	0.733		
	Total	30.8	44			
Beta-sitosterol(mg)	Between Groups	482.311	4	120.578	0.698	0.598
	Within Groups	6906.667	40	172.667		
	Total	7388.978	44			
Vit-A(mcg RAE)	Between Groups	246837.911	4	61709.478	1.304	0.285
	Within Groups	1892481.333	40	47312.033		
	Total	2139319.244	44			
Retinol(mcg)	Between Groups	167114.089	4	41778.522	3.578	0.014*
	Within Groups	467117.111	40	11677.928		
	Total	634231.2	44			
BetaCarotene(mcg)	Between Groups	5172074.917	4	1293018.729	0.332	0.855
	Within Groups	151726023.3	39	3890410.855		
	Total	156898098.3	43			

ANOVA Results for Nutrition Logs for Five Groups cont'd.						
		Sum of Squares	df	Mean Square	F	Sig.
AlphaCarotene(mcg)	Between Groups	276606.356	4	69151.589	0.289	0.883
	Within Groups	9572178.444	40	239304.461		
	Total	9848784.8	44			
BetaCryptoxanthin(mcg)	Between Groups	4724.089	4	1181.022	0.656	0.626
	Within Groups	72029.111	40	1800.728		
	Total	76753.2	44			
Lycopene(mcg)	Between Groups	4514621.644	4	1128655.411	0.359	0.837
	Within Groups	125930766.7	40	3148269.167		
	Total	130445388.3	44			
Lutein+Zeaxanthin(mcg)	Between Groups	1491044.133	4	372761.033	0.222	0.924
	Within Groups	67120765.11	40	1678019.128		
	Total	68611809.24	44			
Vit-A IU	Between Groups	26450557.2	4	6612639.3	0.471	0.756
	Within Groups	561208886.4	40	14030222.16		
	Total	587659443.6	44			
Vit-B1(mg)	Between Groups	2.578	4	0.644	1.611	0.19
	Within Groups	16	40	0.4		
	Total	18.578	44			
Vit-B2(mg)	Between Groups	10.444	4	2.611	1.111	0.365
	Within Groups	94	40	2.35		
	Total	104.444	44			
Vit-B3(mg)	Between Groups	490.533	4	122.633	0.729	0.577
	Within Groups	6728.667	40	168.217		
	Total	7219.2	44			
Vit-B5(mg)	Between Groups	47.467	4	11.867	0.874	0.488
	Within Groups	543.111	40	13.578		
	Total	590.578	44			
Vit-B6(mg)	Between Groups	13.911	4	3.478	1.783	0.151
	Within Groups	78	40	1.95		
	Total	91.911	44			
Choline(mg)	Between Groups	417556.978	4	104389.244	2.659	0.047*
	Within Groups	1570160.222	40	39254.006		
	Total	1987717.2	44			
Folate(mcg)	Between Groups	64572.133	4	16143.033	1.366	0.263
	Within Groups	472581.111	40	11814.528		
	Total	537153.244	44			
FoodFolate(mcg)	Between Groups	26234.356	4	6558.589	0.975	0.432
	Within Groups	269152.222	40	6728.806		
	Total	295386.578	44			
FolicAcid(mcg)	Between Groups	11206.089	4	2801.522	1.121	0.36
	Within Groups	99955.111	40	2498.878		
	Total	111161.2	44			
Dietary Folate Equivalents (mcgDFE)	Between Groups	116245.644	4	29061.411	1.973	0.117
	Within Groups	589134	40	14728.35		
	Total	705379.644	44			
Vit-B12(mcg)	Between Groups	97.778	4	24.444	1.168	0.339
	Within Groups	837.333	40	20.933		
	Total	935.111	44			
Vit-B12, added(mcg)	Between Groups	39.867	4	9.967	1.103	0.368
	Within Groups	361.333	40	9.033		
	Total	401.2	44			
Vit-H(mcg)	Between Groups	29.867	4	7.467	0.636	0.64
	Within Groups	469.333	40	11.733		
	Total	499.2	44			
Vit-C(mg)	Between Groups	7867.422	4	1966.856	0.351	0.842
	Within Groups	224182.222	40	5604.556		
	Total	232049.644	44			
Vit-D(IU)	Between Groups	111671.467	4	27917.867	3.184	0.023*
	Within Groups	350780.444	40	8769.511		
	Total	462451.911	44			
Vit-D2(mcg)	Between Groups	0	4	0	.	.
	Within Groups	0	40	0		
	Total	0	44			
Vit-D3(mcg)	Between Groups	36.756	4	9.189	2.694	0.044*
	Within Groups	136.444	40	3.411		
	Total	173.2	44			

ANOVA Results for Nutrition Logs for Five Groups cont'd.						
		Sum of Squares	df	Mean Square	F	Sig.
Vit-D2+D3(mcg)	Between Groups	50.311	4	12.578	3.681	0.012*
	Within Groups	136.667	40	3.417		
	Total	186.978	44			
AlphaTocopherol(mg)	Between Groups	25.867	4	6.467	1.619	0.188
	Within Groups	159.778	40	3.994		
	Total	185.644	44			
BetaTocopherol(mg)	Between Groups	0.133	4	0.033	0.75	0.564
	Within Groups	1.778	40	0.044		
	Total	1.911	44			
GammaTocopherol(mg)	Between Groups	24.356	4	6.089	2.525	0.056
	Within Groups	96.444	40	2.411		
	Total	120.8	44			
DeltaTocopherol(mg)	Between Groups	1.2	4	0.3	3	0.03*
	Within Groups	4	40	0.1		
	Total	5.2	44			
AlphaTocotrienol(mg)	Between Groups	0.133	4	0.033	0.75	0.564
	Within Groups	1.778	40	0.044		
	Total	1.911	44			
BetaTocotrienol(mg)	Between Groups	0.356	4	0.089	1	0.419
	Within Groups	3.556	40	0.089		
	Total	3.911	44			
GammaTocotrienol(mg)	Between Groups	0.356	4	0.089	1.455	0.234
	Within Groups	2.444	40	0.061		
	Total	2.8	44			
DeltaTocotrienol(mg)	Between Groups	0	4	0	.	.
	Within Groups	0	40	0		
	Total	0	44			
Vit-E(IU)	Between Groups	76.133	4	19.033	0.765	0.554
	Within Groups	994.667	40	24.867		
	Total	1070.8	44			
Vit-E, added(mg)	Between Groups	0.089	4	0.022	1	0.419
	Within Groups	0.889	40	0.022		
	Total	0.978	44			
Vit-K1(mcg)	Between Groups	14949.022	4	3737.256	0.406	0.803
	Within Groups	368502.222	40	9212.556		
	Total	383451.244	44			
Vit-K1D(mcg)	Between Groups	79.422	4	19.856	2.085	0.101
	Within Groups	380.889	40	9.522		
	Total	460.311	44			
Vit-K2(mcg)	Between Groups	11.778	4	2.944	0.805	0.529
	Within Groups	146.222	40	3.656		
	Total	158	44			
Betaine(mg)	Between Groups	12099.778	4	3024.944	1.636	0.184
	Within Groups	73948.667	40	1848.717		
	Total	86048.444	44			
Calcium(mg)	Between Groups	500153.022	4	125038.256	1.295	0.288
	Within Groups	3861558.889	40	96538.972		
	Total	4361711.911	44			
Chloride(mg)	Between Groups	156.8	4	39.2	1	0.419
	Within Groups	1568	40	39.2		
	Total	1724.8	44			
Magnesium(mg)	Between Groups	74232.311	4	18558.078	2.365	0.069
	Within Groups	313820.889	40	7845.522		
	Total	388053.2	44			
Phosphate(mg)	Between Groups	2686979.867	4	671744.967	4.151	0.007*
	Within Groups	6473671.111	40	161841.778		
	Total	9160650.978	44			
Potassium(mg)	Between Groups	4853868.311	4	1213467.078	2.425	0.064
	Within Groups	20016412	40	500410.3		
	Total	24870280.31	44			
Sodium(mg)	Between Groups	14303300.13	4	3575825.033	2.125	0.096
	Within Groups	67313494.67	40	1682837.367		
	Total	81616794.8	44			
Chromium(mcg)	Between Groups	4.756	4	1.189	0.652	0.629
	Within Groups	72.889	40	1.822		
	Total	77.644	44			

ANOVA Results for Nutrition Logs for Five Groups cont'd.						
		Sum of Squares	df	Mean Square	F	Sig.
Copper(mg)	Between Groups	1.778	4	0.444	1.25	0.306
	Within Groups	14.222	40	0.356		
	Total	16	44			
Fluoride(mcg)	Between Groups	91149.111	4	22787.278	0.661	0.623
	Within Groups	1378458.667	40	34461.467		
	Total	1469607.778	44			
Iodine(mcg)	Between Groups	11.556	4	2.889	0.603	0.663
	Within Groups	191.556	40	4.789		
	Total	203.111	44			
Iron(mg)	Between Groups	63.333	4	15.833	0.574	0.683
	Within Groups	1102.667	40	27.567		
	Total	1166	44			
Manganese(mg)	Between Groups	9.911	4	2.478	2.155	0.092
	Within Groups	46	40	1.15		
	Total	55.911	44			
Molybdenum(mcg)	Between Groups	2.089	4	0.522	0.547	0.703
	Within Groups	38.222	40	0.956		
	Total	40.311	44			
Selenium(mcg)	Between Groups	24253.689	4	6063.422	3.665	0.012*
	Within Groups	66175.111	40	1654.378		
	Total	90428.8	44			
Zinc(mg)	Between Groups	74.8	4	18.7	0.677	0.612
	Within Groups	1104.444	40	27.611		
	Total	1179.244	44			
Histidine(g)	Between Groups	9.2	4	2.3	3.09	0.026*
	Within Groups	29.778	40	0.744		
	Total	38.978	44			
Isoleucine(g)	Between Groups	17.778	4	4.444	2.667	0.046*
	Within Groups	66.667	40	1.667		
	Total	84.444	44			
Leucine(g)	Between Groups	54	4	13.5	2.845	0.036*
	Within Groups	189.778	40	4.744		
	Total	243.778	44			
Lysine(g)	Between Groups	51.778	4	12.944	3.058	0.027*
	Within Groups	169.333	40	4.233		
	Total	221.111	44			
Methionine(g)	Between Groups	5.644	4	1.411	2.619	0.049*
	Within Groups	21.556	40	0.539		
	Total	27.2	44			
Phenylalanine(g)	Between Groups	14.889	4	3.722	2.68	0.045*
	Within Groups	55.556	40	1.389		
	Total	70.444	44			
Threonine(g)	Between Groups	15.111	4	3.778	2.636	0.048*
	Within Groups	57.333	40	1.433		
	Total	72.444	44			
Tryptophan(g)	Between Groups	1.244	4	0.311	1.697	0.17
	Within Groups	7.333	40	0.183		
	Total	8.578	44			
Valine(g)	Between Groups	22.8	4	5.7	2.882	0.035*
	Within Groups	79.111	40	1.978		
	Total	101.911	44			
Alanine(g)	Between Groups	25.644	4	6.411	3.223	0.022*
	Within Groups	79.556	40	1.989		
	Total	105.2	44			
Arginine(g)	Between Groups	29.2	4	7.3	2.778	0.04*
	Within Groups	105.111	40	2.628		
	Total	134.311	44			
Aspartic Acid(g)	Between Groups	68.356	4	17.089	2.613	0.05*
	Within Groups	261.556	40	6.539		
	Total	329.911	44			
Cystine(g)	Between Groups	2.756	4	0.689	2.385	0.067
	Within Groups	11.556	40	0.289		
	Total	14.311	44			
Glutamic Acid(g)	Between Groups	251.244	4	62.811	3.206	0.022*
	Within Groups	783.556	40	19.589		
	Total	1034.8	44			

ANOVA Results for Nutrition Logs for Five Groups cont'd.						
		Sum of Squares	df	Mean Square	F	Sig.
Glycine(g)	Between Groups	15.867	4	3.967	3.466	0.016*
	Within Groups	45.778	40	1.144		
	Total	61.644	44			
Hydroxyproline(g)	Between Groups	0.089	4	0.022	1	0.419
	Within Groups	0.889	40	0.022		
	Total	0.978	44			
Proline(g)	Between Groups	24.089	4	6.022	2.765	0.04*
	Within Groups	87.111	40	2.178		
	Total	111.2	44			
Serine(g)	Between Groups	17.333	4	4.333	2.261	0.08
	Within Groups	76.667	40	1.917		
	Total	94	44			
Tyrosine(g)	Between Groups	12.222	4	3.056	3.235	0.022*
	Within Groups	37.778	40	0.944		
	Total	50	44			

Appendix B10 – ANOVA Results for Nutrition Logs of Five Groups. Macronutrients and micronutrients were analyzed with a one-way ANOVA for five groups: LO/DPE, LO/IPE, HI/DPE, HI/IPE, and CON. Significant differences among groups were found for total calories, energy, total calories from carbohydrate, total calories from protein, %calories from protein, total grams of protein, grams protein per kg total body mass per day, grams protein per kg lean mass per day, and micronutrients as indicated (* = $p<0.05$).

11. Tukey Post-Hoc Test for Nutrition Logs of Five Groups

Tukey HSD Post-Hoc Test for Nutrition Logs for Five Groups					
Dependent Variable	(I) GROUP	(J) GROUP	Mean Difference (I-J)	SD	Sig.
Calories	LO/DPE	HI/DPE	-842.22222	289.99651	0.045
		HI/IPE	-968.77778	289.99651	0.015
Energy(kj)	LO/DPE	HI/DPE	-3517.55556	1215.37835	0.046
		HI/IPE	-4050	1215.37835	0.015
Protein(g)	LO/DPE	HI/DPE	-56.88889	15.82064	0.007
		HI/IPE	-59.55556	15.82064	0.005
	LO/IPE	HI/DPE	-56.11111	15.82064	0.008
		HI/IPE	-58.77778	15.82064	0.005
Protein(g/kg)	LO/DPE	HI/DPE	-0.98101	0.19442	<0.001
		HI/IPE	-0.89534	0.19442	<0.001
	LO/IPE	HI/DPE	-0.80914	0.19442	0.001
		HI/IPE	-0.72348	0.19442	0.005
	HI/DPE	CON	0.64665	0.19442	0.015
	HI/IPE	CON	0.56099	0.19442	0.047
Calories/Protein	LO/DPE	HI/DPE	-228.55556	65.1587	0.009
		HI/IPE	-250.11111	65.1587	0.004
	LO/IPE	HI/DPE	-223.33333	65.1587	0.012
		HI/IPE	-244.88889	65.1587	0.005
%Calories/Protein	LO/DPE	LO/IPE	6.88889	2.12016	0.019
	LO/IPE	HI/DPE	-6.55556	2.12016	0.028
Carbohydrates(g)	LO/DPE	LO/IPE	-101.77778	34.99681	0.044
		HI/DPE	-105	34.99681	0.035
		HI/IPE	-116.44444	34.99681	0.015
Estimated Net Carb(g)	LO/DPE	LO/IPE	-110	32.58637	0.013
		HI/DPE	-115.66667	32.58637	0.008
		HI/IPE	-121.44444	32.58637	0.005
		CON	-94.22222	32.58637	0.046
Calories/Carbohydrate	LO/DPE	LO/IPE	-403.11111	132.52144	0.032
		HI/DPE	-420.22222	132.52144	0.023
		HI/IPE	-463.33333	132.52144	0.01
Cholesterol(mg)	LO/DPE	CON	-345.66667	109.09028	0.023
	LO/IPE	CON	-392.11111	109.09028	0.007
Ash(g)	LO/DPE	CON	-6.33333	1.96576	0.02
Retinol(mcg)	LO/DPE	CON	-167.11111	50.94208	0.017
	HI/DPE	CON	-164.55556	50.94208	0.02
Vit-D(IU)	LO/DPE	CON	-141.77778	44.14499	0.021
Vit-D2+D3(mcg)	LO/DPE	CON	-2.88889	0.87135	0.016
	HI/DPE	CON	-2.66667	0.87135	0.03
GammaTocopherol(mg)	LO/DPE	CON	-2.22222	0.73199	0.032
DeltaTocopherol(mg)	LO/DPE	CON	-0.44444	0.14907	0.037
	LO/IPE	CON	-0.44444	0.14907	0.037
Phosphate(mg)	LO/DPE	CON	-670.55556	189.64398	0.009
	LO/IPE	CON	-629.66667	189.64398	0.016
Selenium(mcg)	LO/DPE	CON	-63.66667	19.17393	0.016
	LO/IPE	CON	-59.66667	19.17393	0.027
Isoleucine(g)	LO/IPE	CON	-1.77778	0.60858	0.043
Leucine(g)	LO/IPE	CON	-3	1.0268	0.043
Lysine(g)	LO/IPE	CON	-3	0.96992	0.028
Threonine(g)	LO/IPE	CON	-1.77778	0.56437	0.024
Valine(g)	LO/IPE	CON	-2	0.66295	0.034
Alanine(g)	LO/IPE	CON	-2.11111	0.66481	0.023
Arginine(g)	LO/IPE	CON	-2.33333	0.76417	0.031
Glutamic Acid(g)	LO/DPE	CON	-6.22222	2.08641	0.037
	LO/IPE	CON	-6.44444	2.08641	0.028
Glycine(g)	LO/IPE	CON	-1.55556	0.5043	0.029
Proline(g)	LO/IPE	CON	-2	0.69567	0.048
Tyrosine(g)	LO/IPE	CON	-1.44444	0.45812	0.024

Appendix B11 – Tukey Post-Hoc Test for Nutrition Logs of Five Groups. Macronutrients and micronutrients were analyzed with a one-way ANOVA and Tukey HSD for five groups: LO/DPE, LO/IPE, HI/DPE, HI/IPE, and CON. Table shows all significant relationships ($p<0.05$).

12. ANOVA Results for Total Body Composition

ANOVA Results for Total Body Composition							
			Sum of Squares	df	Mean Square	F	Sig.
% Body Fat	*Baseline*	Between Groups	187.971	4	46.99	1.413	0.247
		Within Groups	1329.933	40	33.25		
		Total	1517.904	44			
	Follow Up	Between Groups	160.065	4	40.02	1.218	0.319
		Within Groups	1314.631	40	32.87		
		Total	1474.696	44			
	Change	Between Groups	3.256	4	0.81	1.083	0.378
		Within Groups	30.073	40	0.75		
		Total	33.330	44			
Lean Mass	*Baseline*	Between Groups	285.726	4	71.43	0.996	0.421
		Within Groups	2869.821	40	71.75		
		Total	3155.547	44			
	Follow Up	Between Groups	303.170	4	75.79	1.112	0.364
		Within Groups	2726.580	40	68.17		
		Total	3029.750	44			
	Change	Between Groups	5.655	4	1.41	1.123	0.359
		Within Groups	50.338	40	1.26		
		Total	55.994	44			

Appendix B12 – ANOVA Results for Total Body Composition. Values at baseline and follow-up and change between the two time points were analyzed with a one-way ANOVA for total body percent fat and lean mass for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). There were no significant differences among groups for any measure at any time point.

13. ANOVA Results for Total Thigh Composition

ANOVA Results for Total Thigh Composition							
			Sum of Squares	df	Mean Square	F	Sig.
Thigh % Fat	*Baseline*	Between Groups	258.477	4	64.619	2.015	0.111
		Within Groups	1283.069	40	32.077		
		Total	1541.546	44			
	Follow Up	Between Groups	239.403	4	59.851	1.797	0.148
		Within Groups	1332.065	40	33.302		
		Total	1571.468	44			
	Change	Between Groups	17.517	4	4.379	4.432	0.005*
		Within Groups	39.524	40	0.988		
		Total	57.041	44			
Thigh Fat Mass	*Baseline*	Between Groups	9670387.022	4	2417596.76	2.944	0.032*
		Within Groups	32845615.28	40	821140.382		
		Total	42516002.3	44			
	Follow Up	Between Groups	8617939.533	4	2154484.88	2.53	0.055
		Within Groups	34064862.94	40	851621.574		
		Total	42682802.48	44			
	%Change	Between Groups	392.77	4	98.193	2.899	0.034*
		Within Groups	1354.926	40	33.873		
		Total	1747.696	44			
Thigh Lean Mass	*Baseline*	Between Groups	7420908.2	4	1855227.05	1.123	0.359
		Within Groups	66079538	40	1651988.45		
		Total	73500446.2	44			
	Follow Up	Between Groups	8423836.3	4	2105959.08	1.248	0.306
		Within Groups	67503189.5	40	1687579.74		
		Total	75927025.8	44			
	%Change	Between Groups	41.922	4	10.481	1.222	0.317
		Within Groups	343.017	40	8.575		
		Total	384.939	44			

Appendix B13 – ANOVA Results for Total Thigh Composition. Values at baseline and follow-up and percent change between the two time points were analyzed with a one-way ANOVA for total thigh percent fat, total thigh fat mass, and total thigh lean mass for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). Significant differences among groups were found for change in thigh percent fat, baseline thigh fat mass, and percent change in thigh fat mass (* = $p<0.05$).

14. ANOVA Results for Thigh Cross Section Composition

ANOVA Results for Thigh Cross Section Composition							
			Sum of Squares	df	Mean Square	F	Sig.
CS % Fat	*Baseline*	Between Groups	244.323	4	61.081	2.321	0.073
		Within Groups	1052.579	40	26.314		
		Total	1296.902	44			
	Follow Up	Between Groups	232.413	4	58.103	2.025	0.109
		Within Groups	1147.579	40	28.689		
		Total	1379.992	44			
	Change	Between Groups	29.301	4	7.325	3.199	0.023*
		Within Groups	91.607	40	2.29		
		Total	120.908	44			
CS Fat Mass	*Baseline*	Between Groups	8031.022	4	2007.756	2.92	0.033*
		Within Groups	27504.556	40	687.614		
		Total	35535.578	44			
	Follow Up	Between Groups	7372.611	4	1843.153	2.479	0.059
		Within Groups	29738.333	40	743.458		
		Total	37110.944	44			
	%Change	Between Groups	1791.1	4	447.775	3.131	0.025*
		Within Groups	5721.068	40	143.027		
		Total	7512.168	44			
CS Lean Mass	*Baseline*	Between Groups	9673.189	4	2418.297	1.366	0.263
		Within Groups	70791.056	40	1769.776		
		Total	80464.244	44			
	Follow Up	Between Groups	8844.144	4	2211.036	1.381	0.258
		Within Groups	64047.556	40	1601.189		
		Total	72891.7	44			
	%Change	Between Groups	33.806	4	8.451	0.776	0.547
		Within Groups	435.631	40	10.891		
		Total	469.437	44			

Appendix B14 – ANOVA Results for Thigh Cross Section Composition. Values at baseline and follow-up and change between the two time points were analyzed with a one-way ANOVA for thigh cross section percent fat, thigh cross section fat mass, and thigh cross section lean mass for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). Significant differences among groups were found for change in thigh cross section percent fat, baseline cross section fat mass, and percent change in cross section fat mass (* = $p<0.05$).

15. Tukey Post-Hoc Test for Body Composition Measures

Tukey HSD Post-Hoc Test for Body Composition Measures					
Dependent Variable	(I) GROUP	(J) GROUP	Mean Difference (I-J)	SD	Sig.
Change Thigh % Fat	LO/DPE	CON	-1.922	0.469	0.002
Thigh Fat Mass Baseline	LO/DPE	HI/DPE	1205.05556	422.16191	0.05
	LO/DPE	HI/IPE	1233.056	427.172	0.047
%Change Thigh Fat Mass	LO/DPE	CON	-9.11161	2.7436	0.016
Cross Section Fat Mass Baseline	LO/DPE	HI/DPE	35.889	12.361	0.045

Appendix B15 – Tukey Post-Hoc Test for Body Composition Measures. All body composition measures were analyzed with a one-way ANOVA and Tukey HSD for five groups: LO/DPE, LO/IPE, HI/DPE, HI/IPE, and CON. Table shows all significant relationships ($p<0.05$).

16. ANOVA Results for Normalized Strength and Power

ANOVA Results for Normalized Strength and Power							
			Sum of Squares	df	Mean Square	F	Sig.
Normalized Isokinetic Flexion	*Baseline*	Between Groups	25.052	4	6.263	2.181	0.092
		Within Groups	97.616	34	2.871		
		Total	122.668	38			
	Follow-Up	Between Groups	13.972	4	3.493	0.873	0.49
		Within Groups	136.058	34	4.002		
		Total	150.03	38			
	%Change	Between Groups	788.733	4	197.183	1.117	0.365
		Within Groups	6004.345	34	176.598		
		Total	6793.079	38			
Normalized Isokinetic Extension	*Baseline*	Between Groups	86	4	21.5	3.028	0.031*
		Within Groups	241.395	34	7.1		
		Total	327.396	38			
	Follow-Up	Between Groups	71.914	4	17.979	1.635	0.188
		Within Groups	373.931	34	10.998		
		Total	445.845	38			
	%Change	Between Groups	973.262	4	243.316	1.863	0.14
		Within Groups	4440.122	34	130.592		
		Total	5413.384	38			
Normalized Isometric	*Baseline*	Between Groups	49.17	4	12.293	0.998	0.422
		Within Groups	418.944	34	12.322		
		Total	468.114	38			
	Follow-Up	Between Groups	54.14	4	13.535	0.705	0.594
		Within Groups	652.771	34	19.199		
		Total	706.911	38			
	%Change	Between Groups	18.396	4	4.599	0.023	0.999
		Within Groups	6699.402	34	197.041		
		Total	6717.798	38			
Normalized Power (Right Leg)	*Baseline*	Between Groups	2373.228	4	593.307	2.527	0.056
		Within Groups	9390.034	40	234.751		
		Total	11763.261	44			
	Follow-Up	Between Groups	2036.95	4	509.238	1.807	0.146
		Within Groups	11270.108	40	281.753		
		Total	13307.059	44			
	%Change	Between Groups	161.103	4	40.276	0.274	0.893
		Within Groups	5875.067	40	146.877		
		Total	6036.17	44			

Appendix B16 – ANOVA Results for Normalized Strength and Power. Values at baseline and follow-up and percent change between the two time points were analyzed with a one-way ANOVA for normalized isokinetic flexion and extension strength (ft-lbs/kg thigh lean mass), isometric strength (ft-lbs/kg thigh lean mass),

and right leg power (Watts/kg thigh lean mass) for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). Significant differences among groups were found for baseline normalize isokinetic knee extension (* = $p<0.05$).

17. ANOVA Results for One Repetition Maximum Testing

ANOVA Results for 1RM Lifts							
			Sum of Squares	df	Mean Square	F	Sig.
Bench	*Baseline*	Between Groups	5047.778	4	1261.944	0.505	0.732
		Within Groups	100022.222	40	2500.556		
		Total	105070	44			
	Follow-Up	Between Groups	7025.556	4	1756.389	0.659	0.624
		Within Groups	106644.444	40	2666.111		
		Total	113670	44			
	%Change	Between Groups	61.563	4	15.391	0.96	0.44
		Within Groups	641.52	40	16.038		
		Total	703.083	44			
Leg Press	*Baseline*	Between Groups	189671.968	4	47417.992	0.825	0.517
		Within Groups	2298150.249	40	57453.756		
		Total	2487822.218	44			
	Follow-Up	Between Groups	631082.031	4	157770.508	1.441	0.238
		Within Groups	4379761.945	40	109494.049		
		Total	5010843.977	44			
	%Change	Between Groups	1056.238	4	264.059	2.254	0.08
		Within Groups	4686.773	40	117.169		
		Total	5743.01	44			
Lat Pull Down	*Baseline*	Between Groups	3781.615	4	945.404	0.498	0.738
		Within Groups	75999.711	40	1899.993		
		Total	79781.326	44			
	Follow-Up	Between Groups	4782.048	4	1195.512	0.518	0.723
		Within Groups	92380.202	40	2309.505		
		Total	97162.25	44			
	%Change	Between Groups	85.115	4	21.279	0.14	0.966
		Within Groups	6061.242	40	151.531		
		Total	6146.358	44			
Knee Extension	*Baseline*	Between Groups	7573.203	4	1893.301	2.322	0.073
		Within Groups	32614.369	40	815.359		
		Total	40187.572	44			
	Follow-Up	Between Groups	16322.93	4	4080.732	3.569	0.014*
		Within Groups	45729.807	40	1143.245		
		Total	62052.736	44			
	%Change	Between Groups	815.528	4	203.882	2.319	0.074
		Within Groups	3517.407	40	87.935		
		Total	4332.935	44			

Appendix B17 – ANOVA Results for One Repetition Maximum Testing. Values at baseline and follow-up and percent change between the two time points were analyzed with a one-way ANOVA for bench press, leg press, lat pull down, and

knee extension 1RM (lbs) for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). Significant differences among groups were found for knee extension 1RM at follow-up (* = $p<0.05$).

18. Tukey Post-Hoc Test for Strength and Power Measures

Tukey HSD Post-Hoc Test for Strength and Power Measures					
Dependent	(I) GROUP	(J) GROUP	Mean Difference (I-J)	SD	Sig.
Normalized Isokinetic Extension - Baseline	LO/IPE	CON	4.11556	1.40435	0.044
	HI/DPE	CON	4.71381	1.48242	0.024
Normalized Power (Right Leg) - Baseline	LO/IPE	CON	21.34556	7.22266	0.039
Knee Extension 1RM - Follow-Up	LO/DPE	CON	56.56333	15.38749	0.006
Knee Extension 1RM - % Change	HI/DPE	CON	58.24444	15.93909	0.006

Appendix B18 – Tukey Post-Hoc Test for Strength and Power Measures. All strength and power measures were analyzed with a one-way ANOVA and Tukey HSD for five groups: LO/DPE, LO/IPE, HI/DPE, HI/IPE, and CON. Table shows all significant relationships ($p<0.05$).

19. Independent Samples Test of FSR for Combined Intervention Groups vs. Control

Independent Samples Test for Combined Intervention vs. Control										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
Samples Comparison	Assumption of Variance	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	SE Difference	95% Confidence Interval of the Difference	
									Lower	*Upper*
FSR INT vs. CON	Equal variances assumed	4.884	0.032*	1.831	43	0.074	12.43165	6.79042	-1.26253	26.12583
	Equal variances not assumed			2.812	29.762	0.009*	12.43165	4.4202	3.40136	21.46194

Appendix 19 – Independent Samples Test of FSR for Combined Intervention Groups vs. Control. Mean myofibrillar fractional synthetic rates (%/day) were analyzed with an independent samples t-test for the combined intervention groups (n=36) compared to control group (n=9). The combined intervention group FSR was significantly greater than control group FSR (* = $p<0.05$).

20. Independent Samples Test of FSR for Individual Intervention Groups vs. Control

Independent Samples Test for Individual Intervention Groups vs. Control										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
Samples Comparison	Assumption of Variance	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	SE Difference	95% Confidence Interval of the Difference	
									Lower	*Upper*
FSR 1:5	Equal variances assumed	14.873	0.001*	2.335	16	0.033*	22.28424	9.54391	2.05206	42.51643
	Equal variances not assumed			2.335	9.672	0.043*	22.28424	9.54391	0.92099	43.6475
FSR 2:5	Equal variances assumed	1.368	0.259	1.704	16	0.108	10.45798	6.13809	-2.55419	23.47015
	Equal variances not assumed			1.704	12.41	0.113	10.45798	6.13809	-2.86694	23.7829
FSR 3:5	Equal variances assumed	7.171	0.017*	1.645	16	0.12	10.5513	6.41599	-3.04999	24.15259
	Equal variances not assumed			1.645	12.003	0.126	10.5513	6.41599	-3.42751	24.53011
FSR 4:5	Equal variances assumed	5.129	0.038*	1.059	16	0.305	6.43308	6.07464	-6.44458	19.31073
	Equal variances not assumed			1.059	12.51	0.31	6.43308	6.07464	-6.74279	19.60895

Appendix 20 – Independent Samples Test of FSR for Individual Intervention Groups vs. Control. Mean myofibrillar fractional synthetic rates (%/day) were analyzed with an independent samples t-test for each intervention group (LO/DPE, LO/IPE, HI/DPE, and HI/IPE) and the control group (CON). The LO/DPE group FSR was significantly greater than control group FSR (* = $p<0.05$).

21. ANOVA Results for FSR of Intervention Groups Normalized to Control

ANOVA Tests of Between-Subjects Effects on FSR for Intervention Groups Normalized to Control							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power (b)
Model	4.862a	3	1.621	1.089	0.368	3.267	0.266
Intercept	112.894	1	112.894	75.867	0	75.867	1
Group PRO	2.148	1	2.148	1.444	0.238	1.444	0.214
Group TIME	2.199	1	2.199	1.478	0.233	1.478	0.218
Group PRO * Group TIME	0.514	1	0.514	0.345	0.561	0.345	0.088
Error	47.618	32	1.488				
Total	165.373	36					
Corrected Total	52.479	35					
a: R Squared = .093 (Adjusted R Squared = .008)							
b: Computed using alpha = 0.05							

Appendix 21 – ANOVA Results for FSR of Intervention Groups Normalized to Control. Myofibrillar fractional synthetic rates normalized to control group means were analyzed with a 2 (low vs. high total protein intake) x 2 (delayed vs. immediate timing of supplementation) ANOVA. There were no significant differences among groups.

22. Interaction Effects of Protein and Timing on FSR

Interaction Effects on FSR of Group PRO and Group TIME					
Group PRO	Group TIME	Mean	SE	95% Confidence Interval	
				Lower Bound	*Upper Bound*
HI	DPE	1.654	0.407	0.826	2.483
	IPE	1.399	0.407	0.571	2.227
LO	DPE	2.382	0.407	1.554	3.21
	IPE	1.648	0.407	0.82	2.477

Appendix 22 – Interaction Effects of Protein and Timing on FSR. Myofibrillar fractional synthetic rates normalized to control group means were analyzed with a 2 (low vs. high total protein intake) x 2 (delayed vs. immediate timing of supplementation) ANOVA. The interaction effect of total intake and timing of supplementation was not significant.

23. Pairwise Comparison of FSR for High and Low Protein Groups

Pairwise Comparisons of FSR for Protein Groups (HI/LO)						
(I) Group PRO	(J) GroupPRO	Mean Difference (I-J)	SE	Sig. (a)	95% Confidence Interval for Difference (a)	
					Lower Bound	*Upper Bound*
HI	LO	-0.489	0.407	0.238	-1.317	0.34
LO	HI	0.489	0.407	0.238	-0.34	1.317
Based on estimated marginal means						
a: Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).						

Appendix 23 – Pairwise Comparison of FSR for High and Low Protein Groups. Myofibrillar fractional synthetic rates normalized to control group means were analyzed with a 2 (low vs. high total protein intake) x 2 (delayed vs. immediate timing of supplementation) ANOVA. There were no significant pairwise comparisons between high and low total protein intake.

24. Pairwise Comparison of FSR for Delayed and Immediate Supplementation Groups

Pairwise Comparisons of FSR for Timing (DPE/IPE)						
(I) Group TIME	(J) Group TIME	Mean Difference (I-J)	SE	Sig. (a)	95% Confidence Interval for Difference (a)	
					Lower Bound	*Upper Bound*
DPE	IPE	0.494	0.407	0.233	-0.334	1.323
IPE	DPE	-0.494	0.407	0.233	-1.323	0.334
Based on estimated marginal means						
a: Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).						

Appendix 24 – Pairwise Comparison of FSR for Delayed and Immediate Supplementation Groups. Myofibrillar fractional synthetic rates normalized to control group means were analyzed with a 2 (low vs. high total protein intake) x 2 (delayed vs. immediate timing of supplementation) ANOVA. There were no significant pairwise comparisons between delayed and immediate protein supplementation.

25. Univariate Tests of FSR for High and Low Protein Groups

Univariate Tests of FSR for Protein Groups (HI/LO)							
	Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power (a)
Contrast	2.148	1	2.148	1.444	0.238	1.444	0.214
Error	47.618	32	1.488				
a: Computed using alpha = 0.05							

Appendix 25 – Univariate Tests of FSR for High and Low Protein Groups. Myofibrillar fractional synthetic rates normalized to control group means were analyzed with a 2 (low vs. high total protein intake) x 2 (delayed vs. immediate timing of supplementation) ANOVA. There were no significant univariate effects of high or low total protein intake.

26. Univariate Tests of FSR for Delayed and Immediate Supplementation Groups

Univariate Tests of FSR for Timing Groups (DPE/IPE)								
	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power (a)
Contrast	2.199	1	2.199	1.478	0.233	0.044	1.478	0.218
Error	47.618	32	1.488					
a: Computed using alpha = 0.05								

Appendix 26 – Univariate Tests of FSR for Delayed and Immediate Supplementation Groups. Myofibrillar fractional synthetic rates normalized to control group means were analyzed with a 2 (low vs. high total protein intake) x 2 (delayed vs. immediate timing of supplementation) ANOVA. There were no significant univariate effects of delayed or immediate protein supplementation.