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The Efficacy of Protein Supplementation on Attenuating Muscle Atrophy Following Disuse in the Collegiate Population

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THE EFFICACY OF PROTEIN SUPPLEMENTATION ON ATTENTUATING

MUSCLE ATROPHY FOLLOWING DISUSE IN THE COLLEGIATE POPULATION

By

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This thesis is approved as a creditable and independent investigation by a

candidate for the Master of Science in Nutritional and Exercise Science degree and is

acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis

does not imply that the conclusions reached by the candidate are necessarily the

conclusions of the major department.

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This thesis is dedicated to my family, for their support and investment in my education and professional aspirations. To my grandfather, for his belief in me and inspiration to excellence. To some of my former student-athletes, for their encouragement on this thesis and confidence in me as their athletic trainer. To God, for His faithfulness.

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ABSTRACT

THE EFFICACY OF PROTEIN SUPPLEMENTATION ON ATTENTUATING

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The purpose of this study is to determine the effects of a protein supplementation protocol through a period of muscular disuse in maintaining muscle mass, strength, jump force production, and cross-sectional area in the collegiate population. Two groups of healthy collegiate participants underwent two weeks of unilateral lower limb suspension (ULLS), during which the control group consumed a normal diet and the treatment group received an additional 75 g of protein supplementation. Lean mass, strength, and force production were measured between dietary intakes at baseline and following immobilization. Muscle area was lost between both groups following ULLS (treatment, -282.8 ± 57.8, p=0.004; control, -349.6 ± 54.8, p=0.001). Between-group analyses did not demonstrate significant results in any of the outcome measures following ULLS; however, there was as trend towards significance in the treatment group of maintaining total lean mass (p=0.08) and leg lean mass (p=0.01) when compared to the control group. Further studies may improve upon the current study design to determine the efficacy of protein supplementation in the active, collegiate population during periods of immobilization with the goal of improving rehabilitation outcomes in this population.

CHAPTER I

INTRODUCTION

Athletes are at an increased risk of injury while participating in sporting events. Data collected by the NCAA Injury Surveillance System has reported that approximately 53% of all injuries that occur from sport-related activities are lower extremity injuries[1]. Alone, ankle ligament sprains and anterior cruciate ligaments (ACL) sprains account for 17.8% of injuries that occur. As a result of orthopedic injuries sustained, many athletes will subsequently require surgical correction and rehabilitation before returning to full participation. The most significant lower extremity injury sustained by NCAA athletes is an ACL injury[1], typically requiring surgical correction. As a result of these ACL reconstructions, many athletes will experience periods of immobilization that will have a multitude of effects at the physiological and metabolic levels.

Following orthopedic injuries and associated surgeries, there is an observed atrophy of involved musculature due to the resulting hormonal and inflammatory response. Oxidative stress increases as proinflammatory cytokines increase following trauma of orthopedic injury, a response which is further elevated following surgical correction [2]. Additionally, several studies have investigated the stress response following inactivity[3,4], suggesting elevated stress hormones[5] in connection to injury and disuse. These responses have been identified as major contributors to protein catabolism and associated loss of muscle mass and strength [2-5]. Gore et al.[5] found an increase in biomarkers of protein breakdown while simultaneously noting a decreased protein

synthesis rate. Simulated hypercortisolemia increased protein catabolism[4,5] in addition to catabolism noted with bed rest alone, suggesting the stress response due to injury and surgery may even magnify the metabolic deficits observed with disuse from orthopedic injury. In addition, muscle atrophy is exacerbated by inactivity during the immobilization period. Several studies have investigated the effects of immobilization and the unweighting of skeletal muscle. Inactivity has been proposed to result in changes in excitation of the spinal cord[6-8], motor unit discharge [9-11], muscle force production [6,7,12], and a negative nitrogen balance [13]. More extensively researched, immobilization has been correlated with loss in muscle mass and strength [2,14-18]. Several lower extremity muscles have been identified as key indicators for ACL injuries, including the quadriceps muscle group, the hamstring muscle group, the gastrocnemius and soleus complex, and the gluteus maximus and medius. Following ACL reconstruction, many studies have demonstrated significant decreases in voluntary activation of the quadriceps [16,18,19]. Similarly, several studies have researched muscle mass difference between involved and uninvolved limbs following surgery, showing significant loss in muscle mass and cross-sectional area related to muscle atrophy following orthopedic injury or immobilization [15,20-23].

Many athletes will be exposed to a rehabilitative protocol to address strengthening from an exercise-based approach, allowing them to utilize neuromuscular stimuli to maintain muscle quality and cross-sectional area following injury. However, there is limited evidence regarding the efficacy of a protein supplementation protocol for this athletic population in order to address the metabolic deficiencies resulting from the hormonal response following injury. Literature [3,24-26] has shown support of protein

supplementation in maintaining protein synthesis following bed rest in the elderly population. In younger populations, Paddon-Jones et al. [27] found 16.5g per day maintained leg mass following 28 days bed rest. But there remains limited evidence for the athletic population in connection with a more applicable form of immobilization seen during rehabilitation. Holm et al. [23] provided ACL patients with supplementation through 18 months, and reported muscle quality and mass gains through 12 weeks of rehabilitation, but found inconsistent results with the nutritional protocol.

The goal of this study is to consider a multi-disciplinary approach to address orthopedic injury and associated surgery, and determine if protein supplementation may have a positive effect on muscle quality and strength in this athletic population.

PURPOSE

The purpose of this study is to determine the effects of a protein supplementation protocol through muscular disuse in maintaining muscle mass, strength, jump force production, and cross-sectional area when compared with a control protocol in the collegiate population between the ages of 18-24.

RESEARCH QUESTION

- Does protein supplementation during immobilization following injury attenuate muscle atrophy that occurs with muscular disuse?

SPECIFIC AIMS

- To determine how protein supplementation affects muscle quality and strength during two weeks of unilateral lower limb suspension (ULLS)

HYPOTHESES

H0: There will be no significant differences in cross-sectional losses following ULLS between those receiving protein supplementation and the control group

H1: ULLS will result in reduced muscle mass and cross-sectional area in both groups, but the treatment group will display a significant reduction in cross-sectional losses

H0: There will be no significant differences in isokinetic quad strength between those receiving protein supplementation and the control group

H2: While both groups will experience a decrease in isokinetic quad strength, the deficit will be greater in the control group than the treatment group

ASSUMPTIONS

- All subjects will display honesty in adherence to unilateral lower limb suspension during the course of the study

- All of the subjects will answer all questions honestly to the best of their ability

- The treatment group will adhere to supplementation protocol during the course of the study

DELIMITATIONS

- All subjects will be students from South Dakota State University

- Subjects will be between the ages of 18-24

- All subjects will participate a minimum of 30 minutes of activity in their daily lives

- No history of a lower extremity orthopedic injury in the past 12 months

- No history of neurological or endocrine disorder

- All subjects in the experimental group will receive the same protein supplementation, Sanford's Profile Perform

LIMITATIONS

- Simulated disuse through ULLS is limited to adherence of the subjects

- Participant requirements of immobilization may limit the number of recruited subjects

- Generalizability of the results to true post-operative patients may be limited due to atrophy from simulated immobilization

CHAPTER II

REVIEW OF LITERATURE

The anterior cruciate ligament (ACL) is the most commonly injured ligament, with between 100,000 and 200,000 ACL tears occurring annually [28]. The ACL runs from the lateral femoral condyle to the intercondylar area of the medial tibial condyle, and assists in the stabilization of the tibiofemoral joint. The ACL has been described in two bundles, the anteromedial and posterolateral bundles, that provide stabilization against anterior translation of the tibia at varying degrees of knee flexion [28,29]. The ACL has been implicated as an important structure on proprioception of the lower extremity due to the number of sensory endings such as Ruffini, Pacinian, and Golgi-like organs found within the structure [28,29]. It is assisted by dynamic support of the surrounding musculature, including those muscles that cross the hip, knee, and lower leg. The most common mechanisms of injury include lateral movements, cutting, twisting, or valgus moments at the knee [28,30,31]. These mechanisms introduce a shear force on the ACL with anterior tibial translation [32]. Studies have shown that the ACL is at a greater risk from 0 to 30 degrees, with the highest load at 15 degrees [30]. Because of the role the ACL plays in stability and proprioception, it has been indicated in injuries of high-demand populations.

ACL Epidemiology

As participation in athletics has increased, there has also been an increase in sport-related injuries. Prior studies indicate that over 50% of all injuries occur in the lower extremity [1,33]. The average ACL incidence was around 36.9 per 100,000 exposures

[34,35]. Within the knee, there are several static and dynamic support structures that are susceptible to injury during athletic participation. Majewski, Susanne, and Klaus [36] reported in a 10 year study that ACL injuries were the most prevalent knee injury accounting for almost half of the knee injuries observed over the study period. Additionally, 80% of knee ligament injuries as claimed by the Accident Compensation Corporation were reported to be injuries to the ACL [34]. The ramifications of ACL injuries can be quite large with an estimated fiscal burden around $11,000 per case for sport-related ACL injuries [34], and over $2 billion dollars annually [37]. In addition, several psychological and physiological impacts may be present post-injury. Following an ACL injury, an athlete may miss 6-9 months of competition while they recover and rehabilitate from surgery, with several weeks possibly being impacted by immobilization and modified weight-bearing status.

There have been several anatomical, biomechanical, neuromuscular, genetic, and hormonal risk factors identified for ACL injuries, specifically non-contact ACL injuries. Increased femoral anteversion, increased Q-angle, tibial internal rotation, subtalar pronation, and a shallow intercondylar notch are several anatomical factors that have been identified to increase the risk of ACL injury [33,37,38]. Several analyses into jump and landing mechanics have identified biomechanical weaknesses that may predispose athletes to ACL injury [32,39]. Upon landing, reduced hip and knee flexion as well as greater knee abduction moments have been shown to put increased stress on the static structures of the knee, including the ACL, rather than allowing proper dispersion of forces through dynamic structures [33,39-43]. One study reported 73% specificity and 78% sensitivity of large knee abduction angles in predicting ACL tears [44]. Increased activation of the

quadriceps has also been proposed as a risk factor for ACL injury, as it relates to the hamstring and quadriceps ratio [30]. The knee extensor muscle group will fire eccentrically to decelerate the leg as the heel strikes during maneuvers such as landing or cutting, and will produce a force often greater than concentric contractions. This force, coupled with reduced hamstring and gastrocnemius activation, will increase load on the ACL and predispose athletes to ACL injury [30,33].

When looking at genetic and hormonal factors, one of the commonly investigated risk factors for ACL injuries is gender-related differences. Walden et al. [45] reported annual prevalence rates of ACL injuries between 0.5-6.0% and 0.5-8.5% for females and males, respectively. Most studies report an average ratio of female to male injuries between 2-3 [46-54]. Gornitsky et al. [55] reported the incidence rate of 81 per 1,000 exposures for females and 50 per 1,000 exposures for males. When number of exposure was controlled, females also displayed a greater incidence of injury in match competitions when compared to males, while the same trend was not identified in practice and training [45]. This gender-related difference has not been identified prior to the age of 12 [45], suggesting a possible correlation with pubescent hormonal changes and increasing risk for females.

Another factor that has been investigated is age-related differences. Beynnon et al. [56] found that college athletes had a significantly higher risk of injury compared to high school athletes (RR= 2.38), when controlled for sport and gender. Similarly, Majewski et al. [36] reported 43.1% of all knee injuries over a 10 year period occurred between the ages of 20-29 when compared to all other age groups, regardless of gender. Another study further delineated ACL injury by age, and reported the highest incidence of ACL injury

for both total and sport-related ACL injuries to be highest in males 20-24 and females 25-29 when compared to other ages [34]. This may be related to the increased activity levels and exposures during this age range. This data may suggest a high-risk population for ACL injuries, and identify a subgroup that may be important to address in rehabilitation.

Physiological Response to Injury

Following an orthopedic injury to the ACL, there is a subsequent physiological response at the cellular and hormonal levels. Pro-inflammatory cytokines such as TNF-α, IFN-γ, IL-1β, IL-6, and IL-8 will increase following trauma [2,57]. This initiates the cellular response of the body to begin the healing process. This inflammatory response has been associated with the muscle atrophy that is observed following disuse and injury, as the circulating cytokines have been linked to several regulating pathways to protein synthesis and protein degradation [2,58-60]. Additionally, this inflammatory response has been observed following ACL reconstruction, suggesting that there is not only an increase in inflammatory cytokines following initial injury, but following reconstructive surgery as well [2]. This prolonged response will initiate a cascade of physiological events that contribute to effects seen after injury and immobilization.

As the resulting cytokines circulate in the body following injury, this will increase oxidative stress of the surrounding cellular environment [2,58]. Oxidative stress will lead to negative protein, lipid, and nitric acid changes [2]. This will lead to an increase in reactive oxidative species (ROS), which has been related to inducing protein breakdown and inhibition of myosin expression [2,59]. Biolo et al. [58] identified three pathways that contribute to degradation of intracellular proteins, including TP-independent lyosomal proteinases, calcium-dependent proteinases, and ATP-dependent requiring ubiquitin.

There is evidence that the ubiquitin-dependent pathway plays a role in myofibrillar degradation when the body shifts into a catabolic state [58,59].

As the rate of protein catabolism increases following oxidative stress of injury, there will also be a simultaneous decrease in protein synthesis. Under normal physiological conditions, the body will undergo mRNA translation to protein through three phases: initiation, elongation, and termination [59]. Following a physiological stress to the system such as injury, protein synthesis is most commonly downregulated by affecting initiation of protein translation. This can be achieved through various pathways. Circulating cytokines such as TNF-α and IL-6 have been shown to affect muscle growth and hypertrophy through the growth hormone (GH) insulin-like growth factor-I (IGF-I) axis [59,60]. Haddad et al.[60] investigated the effects of IL-6 on protein transcription, and proposed a inhibitory interaction of IL-6 on muscle hypertrophy stimulated by GH. In addition, myostatin and NF-kB have been shown to play similar roles in the downregulation of muscle growth [59], altering protein synthesis. Finally, several branch-chain amino acids (BCAA) and other amino acids have been shown to increase protein synthesis under normal conditions, but there is evidence that the amino acid, glutamine, may circulate at lower concentrations in a catabolic state, and therefore fail to stimulate protein synthesis at the same levels [58,61].

Finally, evidence has implicated elevated stress hormones[4,5,62] such as epinephrine, norepinephrine, cortisol and glucagon in altered protein metabolism following injury and disuse. Elevated catecholamines lead to protein catabolism that will contribute to losses in muscle mass and quality [3-5,58]. Waterlow et al. [63] investigated nitrogen balance and amino acid (AA) oxidation following orthopedic surgery, and found

increased AA oxidation led to higher concentration of nitrogen excretion. Following their surgery, patients demonstrated negative nitrogen equilibrium, suggesting surgery may shift the body into a catabolic state. Gore et al. [5] found in increase in biomarker lysine Ra in response to catabolic hormones while simultaneously noting an increase in biomarker lysine Rd, demonstrating increased protein catabolism and decreased protein synthesis, respectively, when the body was introduced to elevated catecholamines. Several studies [4,5] additionally reported an elevated catabolic response following simulated hypercortisolemia. These results may be indicative that the hormone-mediated stress response may increase deleterious effects on muscle mass and quality when compared to disuse alone.

Protein Review

Role of Protein

Protein is a key component of human tissues, including skeletal muscle. Twenty amino acids have been identified as the fundamental units of protein and are classified as nonessential or essential based on the body's ability to synthesize them internally or the requirement of consumption in diet, respectively. Proteins can be found in a variety of sources, including animal, vegetable, and milk proteins. The quality of the protein is determined by the amino acid composition, bioavailability, and digestion [64]. Animal proteins are regarded as high-quality proteins, while vegetable proteins may be incomplete in the amino acid profile, often lacking some essential amino acids. There are some vegetable proteins, such as soy, that contain all the essential amino acids, including the three branch-chain amino acids (BCAA) which have been identified as particularly influential in the regulation of protein synthesis. In addition, another well-known source

of protein comes from whey protein. Whey is taken from milk following the coagulation and curd removal phases and is regarded as a higher quality protein due to the concentration of essential amino acids and BCAAs. Similar to whey, casein protein is found in bovine milk, and also represents a complete protein that also contains calcium and phosphorous [64].

Protein has various functions within the body, including roles in the endocrine, respiratory, neurologic, and digestive systems [61,64]. Proteins are instrumental in the production of hormones, enzymes, and hemoglobin [64]. Protein may also be used as an alternative energy source, when select amino acids are oxidized by branch-chain amino acid dehydrogenase and lead to the production of a derivative of coenzyme A for the citric acid cycle [61]. Because the primary role of protein is to support the growth and maintenance of tissues, protein has been thought to play a key role in maximizing muscle strength and mass.

Protein Response to Injury

Following injury, several physiologic changes occur that affect the structure and function of protein. As discussed previously, the inflammatory and hormonal responses following injury initiate a series of events that shift the body into a catabolic state. This catabolic state is characterized by increased proteolysis [61] as the body breaks down muscle protein in order to sustain the body's metabolic demands. BCAAs are metabolized directly in skeletal muscle and therefore provide an energy source during periods of stress [61]. Glutamine, alanine, and BCAAs may be important in use as substrate during these conditions [58,61] but negatively impact the adequate formation of muscle proteins. An increase in concentrations of free BCAA [61,63,65] indicates a state of muscle

protein catabolism as muscle proteins are broken down [66-70]. This degradation of amino acids for energy subsequently increases the amount of free nitrogen as the amino acids containing nitrogen are broken down [63]. As this nitrogen is excreted, the body shifts into a negative nitrogen balance, indicating that net protein catabolism outweighs net protein synthesis [58,62]. This negative shift in nitrogen homeostasis can happen as quickly as 3 to 4 days post-injury, and has been suggested to plateau at 10 days post-injury [65]. This reaction has been noted to be even more prominent following injury than compared to simple inactivity or starvation alone [71]. As proteins are broken to amino acids, there is an increase in gluconeogenesis [61] as some amino acids are oxidized and converted to an energy source to maintain metabolism. In addition, glutamine concentration will decrease, causing a detrimental effect on cellular metabolism by decreasing nutrient availability to cells [58,61]. Wernerman et al. [62] discussed an additional mechanism, as protein synthesis decreases as proteolysis simultaneously increases. Decreases in protein synthesis rates have been attributed to a 16% decrease in RNA concentration [65] and a 25-30% decrease in ribosome concentration [62] with exposure to increased catecholamines, limiting protein synthesis capacity.

As these conditions simultaneously increase protein catabolism and decrease protein synthesis, muscular atrophy results from the loss of contractile protein. Berg et al. [15] reported several negative consequences to the quadriceps following 6 weeks bedrest, including a 24.5% decrease in isometric torque, a 17.6% decrease in mean fiber area, a 7.8% decrease in fiber diameter, and a 13.8% decrease in CSA. They suggest the greater proportion of strength loss when compared to muscle mass may be related to a greater decline in neural activation following inactivity. Another study has reported a 1.4 kg loss

in muscle mass and a 28.4% decrease in a 1-rep max following inactivity and catecholamine exposure [4]. In addition to these muscular changes, there may be other detrimental effects in response to protein catabolism, such as impaired wound healing, impaired immune function, decreased respiratory function, and decreased coagulation[58].

Rehabilitation Approach

These physiologic changes must be addressed through treatment and rehabilitation following injury in order to improve long-term outcomes. Failing to address the resulting complications from ACL injury and disuse may result in symptomatic changes such as anterior knee pain [72], arthrofibrosis [17], patellofemoral pain syndrome [17], osteoarthritis [73-75], and other degenerative changes [76]. Therefore, it becomes imperative through rehabilitative protocols to address the characteristics of the injured knee such as muscle atrophy, weakness, asymmetry, decreased function, altered biomechanics, and altered neural activation.

Following an ACL injury, several studies have encouraged pre-operative rehabilitation in order to improve long-term outcomes [17,77-79]. Pre-operative restoration of range of motion and quadriceps strength has been shown to result in better post-operative function [78,79], due to decreased motion [80] and strength [17] relating to future complications. Many athletic trainers and physical therapists will incorporate this pre-operative rehabilitation in order to attenuate some of the deleterious effects known to result from the injury response and provide better post-operative outcomes. For this reason, delayed surgical correction may be indicated to better improve patient outcomes [81].

Following surgery, there are also attempts to attenuate the effects of injury and surgical correction. Several studies have looked to define the best post-operative

rehabilitation protocol following ACL reconstruction [77,82-85]. ACL rehabilitation typically consists of three main phases, each to address specific rehabilitative goals. The first phase addresses acute physiologic changes related to minimizing muscle atrophy [77], decreasing pain and effusion [77], reducing arthrogenic muscle inhibition [16], and restoring early motion [17,77,82]. De Carlo et al. [82] emphasized the importance of regaining full motion, reporting better outcomes with an accelerated rehab protocol when compared to a traditional program, showing improved results with more aggressive return of full range of motion early in the recovery. Pre-operative range of motion was significantly related to the restoration of motion following surgery [17], emphasizing the importance of achieving this goal early in post-operative rehabilitation.

When patients achieve these goals, they progress to the second phase of rehabilitation. The goals of this phase are to address the loss of muscle mass in response to the atrophy of injury and surgery. During this phase, patients may begin to focus on restoring strength [77,82-84] in order to establish normal movement patterns, limb symmetry, and weight bearing status [77,83-85]. Myer et al. [83] stressed the importance of regaining full strength in order to reduce the risk of future injury. Deficit in quadriceps strength was identified as a limiting factor in ACL rehabilitation, but they also identified the importance of returning strength through the hip as well. Graft protection is imperative during strength restoration, in order to avoid over-stressing the graft as the joint heals [83]. Butler et al. [86] identified 6-8 weeks post-operative as the weakest points of the graft, necessitating caution when restoring strength and movement during this phase. As patients develop adequate quadriceps strength and dynamic stability of the knee, they may continue to progress through this phase to restore full neuromuscular control.

Strength restoration is essential prior to this progression in order to establish the ability to perform dynamic tasks safely. Adequate strength will allow proper transmission of ground reaction forces during higher intensity activities while protecting the graft [83]. Reductions in knee flexion angles and strength may increase the risk of improper loading and shock absorption [19,87,88]. White et al. [84] studied the effectiveness of perturbation training in the rehabilitation of ACL injuries. They detailed the ACL specialized post-operative return to sports (ACL-SPORTS) training which incorporated prevention exercises, quadriceps strengthening, and perturbations in order to "promote symmetrical joint loading and abate abnormal movement patterns" [84]. Single-leg activities and unstable surfaces are used to develop balance and proprioception, and enhance neuromuscular control to restore normal biomechanics.

The final phase of ACL rehabilitation focuses on functional return-to-play, allowing active individuals to restore full function in higher intensity activities [77]. Full bilateral symmetry through activity-specific movement patterns will be restored. Individuals must be trained to utilize proper biomechanics during activities in order to reduce future risk of injury [77,83]. Adequate strength achieved in prior phases of rehabilitation may become particularly important in active individuals, in order to safely maintain their level of activity when compared to the general population.

Long-Term Indications

There are several indicators that healthcare professionals may utilize in order to determine deficits following injury, as well as to identify successful progression through rehabilitation. The primary indicator has been strength measures. Several studies have identified strength decreases in the presence of muscular atrophy [20-22,89-92]. Because loss

of muscular strength has been related to joint stability [76,83], muscle strength may be an important indicator during the rehabilitative process. This loss of strength is attributed to the atrophy that occurs and losses in muscle mass and cross-sectional area (CSA). In addition to the quadriceps, Norte et al. [21] also studied the muscle mass losses in several muscle groups following ACL reconstruction. They found significant losses in the gracilis, soleus, and gluteus medius, anterior tibialis, and extensor digitorum. This suggests that ACL reconstruction has both distal and proximal effects in regards to muscle mass. This musculature is important in the dynamic function of the knee, and therefore, must also be evaluated in the rehabilitation progression. Similarly, Wall et al. [22] reported quadriceps CSA losses up to 8.4% in just two weeks of disuse. Monitoring CSA and muscle mass is a second indicator that may be used to evaluate progression following ACL injury.

Finally, it has been shown that disuse and atrophy affect neural activation of musculature [6-10,14,89]. Drecshler et al. [18] reported a significant difference in maximum voluntary contraction of the quadriceps in injured knees when compared to uninjured knees at 1 and 3 months post-operation. They also reported a significant relationship between restoration of voluntary activation and strength. Similarly, Urbach et al. [16] also reported a positive correlation between voluntary activation and activity level, suggesting the importance of evaluating the ability to achieve muscular activation following surgery as an indicator for long-term outcomes. Pre-operative isokinetic strength was traditionally thought to be the best indication of post-operative strength outcomes [93], but recent studies have suggested alternative indications that may be better predictors of post-operative outcomes. McHugh et al. [72] suggested that pro-operative EMG frequencies in addition to

strength deficits seen 5 weeks post-operation were the best predictors of strength deficits in the future. Restoration of full strength, muscle mass and CSA, and neural activation of the involved musculature may be important to evaluate when considering the long-term outcomes of ACL rehabilitation.

Protein Supplementation

While it has been well-established that rehabilitation exercises are an effective way to restore muscle mass and strength, there has been some research into additional means of addressing post-operative recovery. In light of understanding the detrimental effect of injury to protein metabolism, it may be beneficial to healthcare professions to address the physiologic mechanism of muscle atrophy during recovery through additional measures. Some studies have investigated the possible benefits of nutritional supplements to attenuate increased protein catabolism and decreased protein synthesis.

Traditionally, protein supplementation has been utilized following activity, as a means of providing increasing availability of amino acids to be used in the formation of protein [64]. Supplementation has been indicated a means of increasing protein synthesis following resistance exercise [64,94-96]. Several studies have reported the benefits of supplementation in connection with training protocols at increasing strength [97-99], improving nitrogen balance [98,100], and preventing loss of lean mass [98]. Protein supplementation was found to be effective at maximally stimulating synthesis while simultaneously delaying catabolism when consumed evenly throughout the day, when compared to a skewed protein intake that may be typical of an average meal plan [101]. As discussed previously, protein can be consumed through a variety of sources, including animal and vegetable. Soy protein is a complete vegetable protein that may provide a

high level of BCAAs [64]. Milk proteins, such as casein and whey, also provide high levels of EAA and BCAAs [64]. Several studies have investigated the difference in protein type on muscle protein synthesis, concluding the protein anabolic response may be dependent on protein type [102-105]. Tang et al. [106] reported better amino acid accumulation with milk proteins when compared with soy. They also found that, while all three protein types induced muscle protein synthesis, whey protein stimulated a greater anabolic response following resistance exercise when compared to soy and casein. They hypothesized this may be due to the higher BCAA—specifically leucine—content of whey protein. Leucine has been shown to be the most effective BCAA at suppressing proteolysis [61,107-109]. It has also been suggested, in addition to high leucine concentration, whey proteins are more rapidly absorbed and have a similar amino acid profile to skeletal muscle [110]. Whey may also provide a high concentration of glutamine [110], which has been shown to increase synthesis when available in the body [61]. By increasing availability of BCAAs in the muscle, there is a decreased breakdown of glutamine as BCAAs provide a nitrogen source for glutamine formation to aid muscle protein synthesis [61]. Overall, it has been well-established that providing EAA and BCAAs will assist in stimulating muscle protein synthesis [106,111,112].

While protein supplementation has been widely studied in healthy populations following exercise, there have been some suggestions that the recommended dietary allowance (RDA) of protein (0.8 g/kg) may not be adequate to prevent a catabolic shift in protein metabolism in certain populations such as the elderly, ill, physically inactive, or injured populations [4,24,113-115]. In response, there has been a shift in research to investigate the possibility of protein supplementation to counteract these catabolic states. Several

studies that have investigated the use of protein supplementation in elderly populations [3,25,26,114,116], supporting the hypothesis that increased availability of EAAs through supplementation stimulates protein anabolism. Ferrando et al. [3] found maintenance of lower extremity strength tasks in those with EAA supplementation during a period of bed rest when compared to those without. By providing supplementation, this allows the body to counteract the negative protein balance by increasing turnover and providing substrates for muscle protein synthesis [3]. There have been similar results found in younger populations. Paddon-Jones et al. [27] found that EAA supplementation maintained muscle mass and strength in healthy young adults following bed rest. In addition to EAA and BCAA content, studies have investigated the multiplicative effects of carbohydrate content in addition to EAAs, demonstrating an increase in synthesis due to the body's insulin response to glucose, which further stimulates protein synthesis in the body [23,27,117-120]. Holm et al. [23] found some strength gains following EAA and carbohydrate supplementation after rehabilitation sessions in ACL patients, although they reported some inconsistent results with the protocol through the population.

While several of these studies focus on the isolated effects of protein supplementation in addressing the physiologic deficits following injury and immobilization, it has also been suggested that supplementation alone will not be adequate in fully maintaining muscle mass without a neuromuscular stimulus [27]. There are very limited studies investigating the possibility of a multidisciplinary approach to post-operative recovery, incorporating both rehabilitative exercise and protein supplementation. In addition, while most of the evidence supports the use of supplementation in the elderly population, there remains a lack of conclusive evidence

evaluating the efficacy of supplementation in meeting the metabolic requirements of younger, active populations. Because data has shown a high incidence of injuries such as ACL tears in this population, it may be relevant to investigate the possible efficacy of protein supplementation in the collegiate population.

CHAPTER III

METHODOLOGY

Subjects

Fourteen individuals ages 18-24 were recruited from the student population of South Dakota State University. Subjects were classified as active individuals based on a reported minimum of 30 minutes activity on at least three days a week. Subjects were randomly assigned to the control and treatment groups. Subjects completed a medical history questionnaire (Appendix A) to exclude any subjects with a history of lower extremity orthopedic injury in the previous 12 months, neurological disorders, or endocrine disorders. At this time, all subjects were fully informed of the nature of the study and possible risks and completed written informed consent. Subjects also completed an enrollment form and a physical activity readiness questionnaire (Appendix B, C).

Diet and Physical Activity

All subjects were educated on adhering to unilateral lower limb suspension at all times during the course of the study. Subjects were encouraged to remain non-weight bearing during showering and during the range of motion exercises prescribed by the investigators for the study. During the course of the study, the control group was educated to continue their normal diet and refrain from supplementation through the entirety of the study. Additionally, all subjects assigned to the treatment group were given Sanford's Profile Perform supplement (Profile Restore Protein Blend [micellar

casein, whey protein concentrate, whey protein isolate], Appendix D) providing 25g protein, 20g carbohydrates per serving, for three servings per day.

Procedures

At the first study visit, subjects were randomly assigned to treatment or control group. Each subject completed all questionnaires and obtained baseline outcome measurements. Dual-energy x-ray absorptiometry (DEXA) was used to evaluate body composition. Peripheral computed tomography (pQCT) was performed to assess total bone cross sectional area of the tibia, cortical volumetric bone mineral density, muscle cross sectional area, and muscle density. The Biodex System 4 Dynamometer was utilized to assess isokinetic muscle strength of the quadriceps. The subjects seated and strapped to the chair at their shoulders and waist, and their foot was secured to the holder with a similar strap. Each subject was allowed a series of familiarization repetitions. Each performance trial consisted of five maximal effort isokinetic quadriceps extension repetitions at 60 degrees per second, 180 degrees per second, 300 degrees per second. Each series was separated by 60 seconds rest. Ground reaction force and movement efficiency were measured using a Leonoardo Mechanograph. The first test consisted of the participant standing on the force platform and jumping as high as they could one time on both feet. The participant received no instruction other than to jump as high as they could and to be still after they land. This was repeated three times and the highest measurements from the three jumps was used. The next test consisted of the participant performing a maximal one-legged jump. This test was performed bilaterally with the participant repeating the test three times per leg. Treatment group subjects were then instructed to begin protein supplementation.

All subjects were fitted with a Don-Joy TROM Advanced knee brace set to allow 90 degrees of flexion and restricting 30 degrees of extension. Participants were fitted with a knee walker and were allowed to bend to 90 degrees while utilizing the knee walker. All subjects were educated to begin unilateral lower limb suspension for two weeks. All subjects were given non-weight bearing range of motion exercises to complete during the two weeks to simulate post-operative rehabilitation. Following the two weeks of immobilization, all participants were re-evaluated for all outcome measures.

Data Analysis

Between-group differences at baseline were analyzed using Student's t-tests. Changes in outcome variables within group were tested for significance using a paired t-test. Finally, linear regression models adjusting for baseline value of the outcome measurement and baseline weight were used to determine if the changes observed were different between the treatment and control groups.

CHAPTER IV

RESULTS

Baseline Characteristics

Baseline characteristics are shown by group in Table 1. At baseline, the groups

were not different in any of the measurements. It should be noted that while body weight

was not significantly different between groups, there was an 11-kilogram difference

between the treatment and control groups (75.0 ± 22.0 kg and 86.1 ± 23.4 kg,

respectively). Due to the known association between body weight and the outcome

measurements, weight was included as a covariate in regression models.

Change in Outcome Variables

Changes in outcome variables are given by group in Table 2. An expected

finding from this study was that participants in the treatment group increased their peak

torque production in their control leg at both 180 and 300 degrees per second (12.2 ± 3.2

nM and 8.1 ± 2.1 nM, respectively, p=0.01, both). Participants in the treatment group

gained significant lean mass during the study (1.02 ± 0.37 kg, p=0.04) while the control

group did not change (-0.01 ± 0.39 kg, p=0.97). Both the treatment and control groups

lost muscle in their affected leg during the study (-282.8 ± 57.8 mm^2, p=0.004 and -349.6

± 54.8 mm^2, p=0.001, respectively).

Group Differences in the Change in Outcome Measurements

None of the changes in outcome measures were significantly different between

the treatment and control groups. However, change in lean mass indicated a trend toward

Table 1. Baseline Measurements

	Treatment	Control	P-Value
Height (cm)	173.9 ± 15.9	174.5 ± 7.7	0.929
Weight (kg)	75.0 ± 22.0	86.1 ± 23.4	0.385
Percent Fat (%)	24.5 ± 5.2	28.4 ± 7.9	0.321
Lean Mass (kg)	57.0 ± 16.1	61.3 ± 14.3	0.604
Fat Mass (kg)	18.7 ± 8.2	25.3 ± 11.4	0.253
Affected Leg Lean Mass (kg)	8.7 ± 2.1	9.8 ± 2.3	0.369
Control Leg Lean Mass (kg)	8.8 ± 2.3	9.7 ± 2.3	0.516
Affected Calf Muscle Area (mm^2)	7221 ± 2031	7361 ± 1767	0.893
Control Calf Muscle Area (mm^2)	7260 ± 1962	7256 ± 1649	0.997
Affected Jump Force	1.5 ± 0.4	1.7 ± 0.6	0.478
Control Jump Force	1.5 ± 0.4	1.7 ± 0.5	0.523
Affected Peak Torque 60 deg/sec	129.0 ± 43.1	126.3 ± 25.6	0.893
Control Peak Torque 60 deg/sec	136.0 ± 51.8	122.7 ± 23.0	0.552
Affected Peak Torque 180 deg/sec	83.1 ± 28.8	85.2 ± 21.8	0.855
Control Peak Torque 180 deg/sec	80.4 ± 26.3	80.2 ± 19.9	0.983
Affected Peak Torque 300 deg/sec	60.3 ± 19.6	62.9 ± 16.2	0.797
Control Peak Torque 300 deg/sec	64.4 ± 23.3	62.6 ± 12.9	0.861

Data are given as means ± SD
p-values indicate the difference between groups

significance (p=0.08) with the treatment group having an increase while the control group

had a small decrease in lean mass (Figure 1). Similarly, there was a trend toward

significance in leg lean mass (p=0.1) with the treatment group showing an increase while

the control group had a loss in leg lean mass (Figure 2). These trends were investigated

further using post-hoc power analyses and it was determined that for each of these trends,

a sample size of 15 per group would yield 80 percent power to detect a difference at a

0.05 significance level.

Table 2. Change in Outcome Variables

	Treatment	p-Value	Control	p-Value
Percent Fat (%)	-0.59 ± 0.39	0.20	-0.14 ± 0.41	0.75
Lean Mass (kg)	1.02 ± 0.37	0.04	-0.01 ± 0.39	0.97
Fat Mass (kg)	-0.33 ± 0.38	0.42	0.01 ± 0.40	0.99
Affected Leg Lean (kg)	0.20 ± 0.16	0.26	-0.39 ± 0.26	0.18
Control Leg Lean (kg)	0.35 ± 0.17	0.10	-0.04 ± 0.23	0.87
Affected Calf Muscle Area (mm^2)	-282.8 ± 57.8	0.004	-349.6 ± 54.8	0.001
Control Calf Muscle Area (mm^2)	184.9 ± 106.1	0.14	54.6 ± 65.3	0.43
Affected Jump Force	-0.03 ± 0.03	0.40	-0.11 ± 0.05	0.07
Control Jump Force	-0.01± 0.11	0.91	-0.11 ± 0.13	0.40
Affected Peak Torque 60 deg/sec	-7.9 ± 6.6	0.29	-11.1 ± 5.9	0.11
Control Peak Torque 60 deg/sec	-4.1 ± 5.4	0.48	14.1 ± 9.1	0.17
Affected Peak Torque 180 deg/sec	-2.1 ± 2.6	0.45	-1.9 ± 3.7	0.63
Control Peak Torque 180 deg/sec	12.2 ± 3.2	0.01	13.5 ± 6.0	0.06
Affected Peak Torque 300 deg/sec	5.1 ± 4.3	0.29	2.1 ± 5.1	0.70
Control Peak Torque 300 deg/sec	8.1 ± 2.1	0.01	8.8 ± 4.9	0.12

p-values indicate the difference between baseline and post

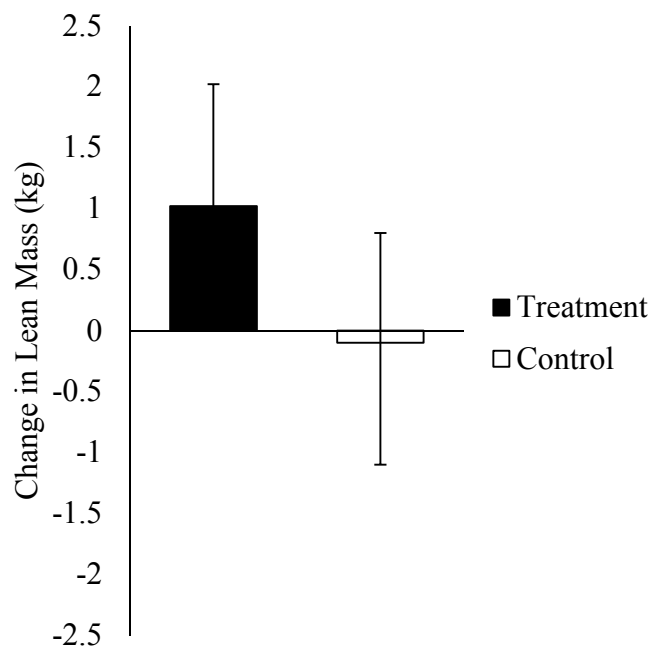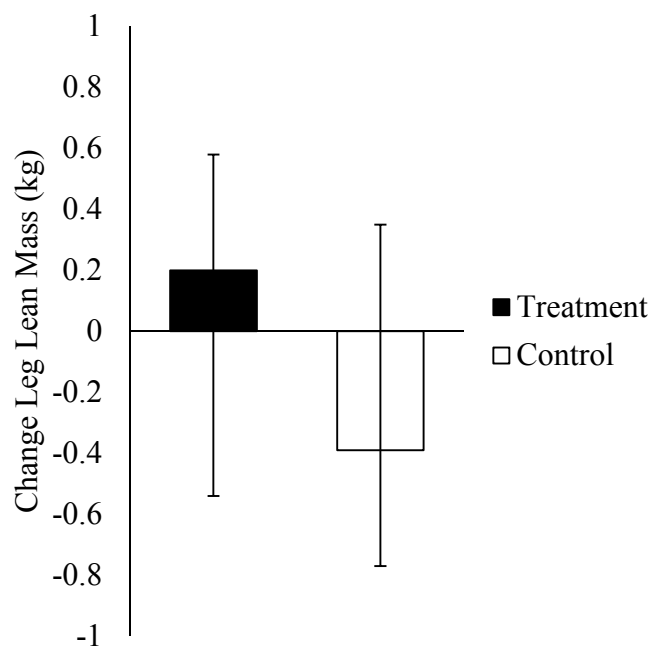Figure 1. Change in Lean Mass Between Groups

Figure 2. Change in Leg Lean Mass in the Affected Leg between Groups

CHAPTER V

DISCUSSION

The main goal of this study was to determine the effects of protein

supplementation on maintaining muscle mass and quality during periods of disuse

simulated with ULLS. While many studies have investigated the effects of various means

of immobilization [12,14,15,22,89,90] on a variety of functional outcome measures, the present

study sought to determine the impact of a nutritional intervention while immobilized.

We hypothesized that ULLS would result in a decrease in muscle mass, as

supported by previous studies' findings regarding periods of immobilization [15,22,65,89].

While some studies have used bedrest for prolonged periods of time when studying

changes to protein synthesis and muscle quality[15,27], Wall et al. [22] has previously

demonstrated that short-term immobilization periods such as two weeks can also result in

substantial changes. They noted a decrease in quadriceps CSA of 8.4% over the two-

week period. Research has reported an approximate 3% loss in muscle mass per week

during periods of disuse[15]. This loss of muscle mass has been related to a decrease in

muscle protein synthesis, with a concomitant increase in proteolysis. Loss in muscle CSA

and volume can be expected within injured populations as well, with extensive literature

supporting muscle mass loss in ACL patients in both preoperative and postoperative

states[18,20,21,121]. Various studies have measured this in CSA of several key muscles in

lower limb stability and strength. Berg et al. [15] reported a decrease in mean fiber area and

diameter of the knee extensors following bedrest. The present study found no significant

deficit in total body and leg lean mass in the control group, while the treatment group displayed an overall increase in both total and leg lean mass, contradicting the prior research. This finding may be explained by the means of immobilization used in the present study. Heitkamp et al. [122] identified early strength gains may be related to neural adaptations, particularly after balance training. The present study utilized knee walkers as the means of immobilization, requiring single limb stance on the contralateral limb during stance phase, while also requiring a single knee stance during the contralateral swing phase of gait. This means of immobilization differs from prior studies. Previous research [123] has discussed the possibility of cross-over effects from balance training, suggesting the adaptations that occur at the neural level with perturbation training of one limb while present with improved neuromuscular control on the contralateral limb. With the neuromuscular stimulus provided during stance phase of the contralateral limb as well as the single knee stance of the ipsilateral leg during gait may reduce the potential effects of immobilization and may explain why the data did not reflect the same degree of muscle mass loss as previous literature has found. It may be possible the contralateral balance training of single limb stance during gait with the knee walker may have maintained the neural response for the immobilized limb even during the two-weeks of the ULLS, presenting with a nonsignificant change in the control group while the protein supplementation may have promoted an overall increase without the expected deficit with immobilization.

Conversely, the present study did note an equivalent decrease in calf muscle area in both groups. Literature [14,15,89] has indicated that certain fiber types such as type I fibers may be more prone to atrophy following immobilization, resulting in decreased type I

fiber diameter following unloading. These results may support that a common muscle group consisting of predominantly type I fibers, such as the calf, may be more inclined to atrophy when compared to the knee extensor mass. Similarly, the means of ULLS may explain the muscle area change observed in the present data. While a higher neuromuscular stimulus may be noted in the ipsilateral knee and hip during the stance phase requiring a single knee stance, the involved calf was not presented with the same potential neural stimulus during immobilization in the present study.

We also hypothesized a concomitant strength loss from baseline in both groups, while expecting a greater deficit in the control group. Strength losses up to 4-5% per week have been reported [15] during periods of disuse. During immobilization, various muscle groups may be impacted. Previous literature has focused on the knee extensors and plantar flexors, due to their use during ambulation and knee stability; Clark et al. [14] reported a 15% and 25% decrease in plantar flexors and knee extensors, respectively, following four weeks of unloading. Post-injury, similar results have been established in strength losses, with prolonged muscle loss being an important consideration in rehabilitation goals[18,21,72,124]. Conversely, the present study did not find any significant deficits in peak torque production from baseline, nor any significant between-group differences following immobilization. As previously mentioned, maintenance of muscle strength during ULLS may be attributable to the method of ULLS and the maintenance of neuromuscular stimuli to the muscle. It has been established that early positive adaptations to strength training are the result of central nervous system adaptations [125], and research has also concluded that similar neural adaptations play a role in the loss of strength following immobilization [126,127]. Heitkamp et al. [122] reported an increase in both

knee flexors and extensors with balance training, similar to gains made of the control group undergoing traditional strength training. Additionally, we found no significant differences in jump force between groups, which may be explained by the maintenance of motor recruitment and neuromuscular control, thereby reducing the expected loss of both strength and function in both groups.

Previous literature has indicated the relationship between outcome measures of immobilization and muscle protein synthesis within the body [4,27,65], establishing the simultaneous increase in protein catabolism and decrease in protein synthesis. The overall deleterious effect produces the expected outcomes in muscle mass, strength, and activation loss. To address these physiologic changes, many studies have looked at nutritional interventions to supplement traditional rehabilitation strategies. Paddon-Jones et al. [27] reported significant attenuation in leg muscle mass and strength losses with the consumption of essential amino acid-carbohydrate (EAAC) supplement during bedrest. While they reported that the EAAC intervention was not sufficient alone to maintain muscle mass without a neuromuscular stimulus, they did note stimulated protein synthesis. These findings have previously been supported by literature [3,26,128,129], suggesting that supplementation has positive effects on protein synthesis rates. While research has not established a specific supplementation protocol, studies have looked to determine the best supplementation to provide, noting that EAAs—specifically BCAAs—have the most stimulatory effect on protein synthesis [3,27,110]. Additionally, many studies [27,117,119,130,131] have investigated the effects of adding carbohydrates to supplementation, with beneficial results. This is due to the increase in insulin secretion in response to carbohydrate ingestion that will subsequently promote further protein

synthesis. This overall maintenance of protein synthesis and nitrogen balance, particularly during periods of immobilization and injury, due to EAAC supplementation has been previously researched in the elderly [3,116,120,128], the young [27,65,106], and injured populations [23]. The present study did not demonstrate comparable results to previous literature, as there was no significant between-group difference in leg lean mass following protein supplementation. However, it may be noted that the current study found that those receiving supplementation displayed an attenuated decrease in lean mass that was trending towards significance ($p = 0.06$, Figure 1) when compared to the control group. These data support previous research citing supplementation as a possible means of attenuation. Additionally, we discussed earlier possible effects of supplementation on the treatment group, showing an overall increase in both total lean mass and leg lean mass from baseline. EAA ingestion has been shown to increase net muscle protein synthesis when combined with a stimulus such as exercise [94]. With maintained neuromuscular stimulus during this study as previously discussed, the addition of EAA to the treatment group intake may promote an increase in muscle protein synthesis that could explain the changes in lean mass demonstrated by this study.

Limitations

The present study had a small sample size, and therefore limited generalizability of results. Future research may build upon these results by increasing participation or by investigating different populations. The current study selected active collegiate participants within the general population for athletic implications and rehabilitation protocols; future studies may look specifically at athletes to determine the effect of immobilization.

As discussed previously, the present study also utilized a different form of immobilization when compared to prior research. This means of immobilization may be an important factor in achieving expected results of atrophy and muscle disuse in order to further establish the effect of supplementation. Hackney & Ploutz-Snyder[127] reviewed ULLS and its associated mechanisms for atrophy; they determined alterations in the neuromuscular stimuli during periods of ULLS included decreases in motor unit recruitment and EMG activity. The present means of ULLS may not provide the same cessation of neuromuscular stimuli to induce the expected atrophy, as discussed earlier. The present means of ULLS was utilized due to difficulty in participant recruitment, and further studies may be able to recruit adequate participants with traditional means of ULLS. In addition, further research may also include outcome measures to gauge the nervous system response to ULL, including EMG data. By providing feedback related to motor unit recruitment or electrical activity, further studies may be able to measure the impact on the neurological mechanisms associated with atrophy.

Furthermore, determination of participants' full adherence to the immobilization protocol could not be determined beyond self-reported adherence, which could potentially impact atrophy. Prior studies have utilized an accelerometer [6,132] in order to estimate patient compliance. This could assist in an accurate representation of subject compliance with the protocol to support the results.

Additionally, further studies should investigate the effects of supplementation within these athletic populations when used in connection with rehabilitative protocols. As this current data may demonstrate along with prior research, the presence of neuromuscular stimuli may help maintain muscle mass during periods of disuse. Within

the athletic population, it may be investigated the additional effects of supplementation with outcome-oriented rehabilitation to achieve higher muscle mass, strength, and function goals related to athletic return to sport. This study may serve as a pilot to continue building towards evidence for a multi-disciplinary approach to athletic rehabilitation.

Conclusion

The specific aim of this study was to determine how protein supplementation affects muscle quality and strength during and following two weeks of ULLS. The present study did not find the hypothesized deficits in total lean mass or leg lean mass following suspension. Similarly, we concluded no significant strength or function deficits in either group with ULLS. However, the current study found that protein supplementation may have positive impacts on body composition, with a significant increase in total lean mass in those receiving supplementation following immobilization, trending towards significance when compared to the lean mass lost by those not receiving supplementation.

APPENDICES

APPENDIX A

<u>MEDICAL HISTORY FORM</u>

Name_____   Participant ID_____   Date_____

Are you currently taking any medications (initial visit only)?   YES   NO

> If Yes, please list below.

Medication	Reason

Medical Details

Do you have any of the following conditions (*Check all that apply)*

	Acid Reflux (Heartburn)		Chronic Low Back Pain
	Alcoholism		Depression
	Allergies		Diabetes Type 1
	Anxiety		Diabetes Type 2
	Asthma		High Blood Pressure
	Atrial Fibrillation		Irritable Bowel Syndrome
	Cancer		Migraines
	Bleeding/Clotting Problem		Low Bone Density or Osteoporosis
	High Cholesterol		Thyroid Problem
	Heart Disease		Kidney Disease
	Blood Clots		Osteoarthritis
	Tuberculosis		Rheumatoid Arthritis

	Emphysema		Anemia
	Hernia		Muscular Dystrophy

Do you have any of the following conditions (*Check all that apply)*

	Have you ever had an injury to a bone, muscle, ligament, or tendon that required treatment?	Explain:
	Have you ever broken any bones or dislocated joints?	Explain:
	Have you ever had a stress fracture?	Explain:
	Do you regularly use a brace, orthotics, or other assistive device?	Explain:
	Do you have a bone, muscle, or joint injury that bothers you?	Explain:
	Do you have any history of arthritis or connective tissue disease?	Explain:

Please list all surgeries you have had in the past 5 years:

Surgery	*Year*

APPENDIX B

ENROLLMENT FORM

Name:_____Enrollment Date:_____Participant ID:_____

Phone Number:_____Email Address:_____DOB:_____

Does the study staff have permission to send email messages to remind you of upcoming visits?

YES   NO

Does the study staff have permission to send text messages to remind you of upcoming visits?

YES   NO

Race:

White   Black   American Indian/Alaska Native

Asian or Pacific Islander   Unknown

Height:_____Weight:_____BMI:_____

Have you suffered any lower extremity injury in the past 12 months?   YES   NO

How many days per week do you perform at least 30 minutes of physical activity?

_____

APPENDIX C

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q)

Name:_____Date:_____

	Questions	Yes	No
1	Has your doctor ever said that you have a heart condition and that you should only perform physical activity recommended by a doctor?		
2	Do you feel pain in your chest when you perform physical activity?		
3	In the past month, have you had chest pain when you were not performing any physical activity?		
4	Do you lose your balance because of dizziness or do you ever lose consciousness?		
5	Do you have a bone or joint problem that could be made worse by a change in your physical activity?		
6	Is your doctor currently prescribing any medication for your blood pressure or for a heart condition?		
7	Do you have ANY other reason why you should not engage in physical activity?		

APPENDIX D

Nutrition Facts

20 servings per container

Serving size	2 Scoops (54g)

Amount per serving

Calories 190

	% Daily Value*
Total Fat 2g	3%
Saturated Fat 1g	5%
Trans Fat 0g	
Cholesterol 20mg	7%
Sodium 260mg	11%
Total Carbohydrate 20g	7%
Dietary Fiber 1g	4%
Total Sugars 13g	
Includes 10g Added Sugars	20%
Protein 25g	50%

Vitamin D 4mcg 20%	• Calcium 450mg 35%
Iron 4mg 20%	• Potassium 400mg 8%
Vitamin A 180mcg 20%	• Vitamin C 27mg 30%
Vitamin E 3mg 20%	• Vitamin K 24mcg 20%
Thiamin 0.4mg 30%	• Riboflavin 0.4mg 30%
Niacin 3.2mg 20%	• Vitamin B_6 0.5mg 30%
Folate 80mcg DFE 20%	• Vitamin B_{12} 0.5mcg 20%
(48mcg folic acid)	• Biotin 6mcg 20%
Pantothenic Acid 1mg 20%	• Phosphorus 310mg 25%
Iodine 30mcg 20%	• Magnesium 150mg 35%
Zinc 2.2mg 20%	• Selenium 11mcg 20%
Copper 0.2mg 20%	• Manganese 0.5mg 20%
Chromium 7mcg 20%	• Molybdenum 9mcg 20%

*The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Calories per Gram		
Fat 9 •	Carbohydrate 4 •	Protein 4

Ingredients:
Profile Restore Protein Blend (Micellar Casein, Whey Protein Concentrate, Whey Protein Isolate), Sugar, Cocoa, Fructose, Natural Flavor, Isomaltulose, Nonfat Milk, L-Glutamine, Resistant Maltodextrin, Cornstarch, Salt, Guar Gum, Cocoa Extract, Medium Chain Triglycerides, Xanthan Gum, Maltodextrin, Soy Lecithin, Whey, Sucralose, Carrageenan, Modified Cornstarch, Bromelain, Probiotics (*Bacillus coagulans* GBI-30 6086), Gum Arabic, Papain, Microcrystalline Cellulose.

Vitamin and Mineral Blend:
Tricalcium Phosphate, Magnesium Oxide, Dipotassium Phosphate, Vitamin C, Ferric Orthophosphate, Vitamin E [Alpha Tocopherol Acetate], Zinc Sulfate, Niacinamide, Copper Gluconate, Manganese Sulfate, D-Calcium Pantothenate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Thiamin Mononitrate, Chromium Nicotinate Glycinate Chelate, Selenium Glycinate Complex, Folic Acid, Molybdenum Glycinate Chelate, Potassium Iodide, Vitamin K [Phytonadione], Biotin, Magnesium Carbonate, Vitamin D_3, Vitamin B_{12} [Cyanocobalamin].

Contains: Milk and Soy Lecithin.

Includes Isomaltulose, a very low glycemic carbohydrate which provides sustained energy release.

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