The Effects of Pre-Sleep Dairy-or Plant-Based Protein Consumption on Muscle Recovery Following Morning Eccentric Exercise in Middle-Aged Men

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THE EFFECTS OF PRE-SLEEP DAIRY- OR PLANT-BASED PROTEIN CONSUMPTION ON MUSCLE RECOVERY FOLLOWING MORNING ECCENTRIC EXERCISE IN MIDDLE-AGED MEN

By

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ABSTRACT

Pre-sleep protein has been shown to improve overnight muscle protein synthesis, muscle size and strength, and muscle recovery. However, all pre-sleep protein studies to date have utilized dairy-based protein sources. There is currently a large interest in alternative protein sources, such as plant-based protein. Yet, there is no evidence regarding the efficacy of plant-based proteins consumed pre-sleep.

PURPOSE: Therefore, the purpose of this study was to compare animal-based vs. plant-based pre-sleep protein on muscle recovery as measured by blood markers of muscle damage and inflammation, metabolism, and muscle function.

METHODS: Twenty-seven males performed a bout of eccentric exercise (ECC) for the knee extensors (ext) and flexors (flex) in the morning, then consumed 40 g of either whey hydrolysate (WH, n = 9), whey isolate (WI, n = 6), rice and pea combination (RP, n = 6), or placebo (PL, n = 6) 30 min pre-sleep. The ECC protocol consisted of 5 sets of 15 repetitions of maximal eccentric voluntary contractions for ext and flex of each leg, respectively. Catered meals (15% PRO, 55% CHO, 30% Fat) were provided to participants two days pre-ECC, the day of ECC, and the following two days during follow-up testing to standardized nutrition. Plasma creatine kinase (CK), interleukin-6 (IL-6), interleukin-10 (IL-10), and C-reactive protein (CRP) was measure at pre, post, +4, +6, +24, +48, and +72-hrs post-ECC. Isometric and isokinetic maximal voluntary contraction (ISOM and ISOK, respectively) was measured at pre, post, +24, +48, and +72-hrs post-ECC. Subjective muscle soreness, thigh circumference, and HOMA-IR was measured at pre, +24, +48, and +72-hrs post-ECC.

RESULTS: CK increased at +4-hrs post-ECC and remained elevated at all time points compared to baseline ($n = 25$, $p < 0.001$) and was significantly greater at +72-hrs compared to all other time points ($p < 0.001$). IL-6 increased at +6-hrs ($n = 25$, $p = 0.002$) with no other time differing from baseline. CRP increased immediately post-ECC ($n = 25$, $p = 0.035$) with no other time differing from baseline. There were no group x time interactions for any blood marker. ISOMext was reduced after ECC ($p = 0.001$) and remained reduced until returning to baseline at +72 hrs. ISOMflex was reduced after ECC and remained reduced at +72-hrs ($p < 0.001$). ISOK60ext and ISOK60flex were reduced after ECC and remained reduced at +72-hrs ($p < 0.001$). ISOK180ext and ISOK180flex were reduced after ECC and remained reduced at +72-hrs ($p < 0.05$).

ISOK300ext was reduced after ECC ($p < 0.05$) and remained reduced until returning to baseline at +72 hrs. ISOK300flex was reduced at +24-hrs and remained reduced at +72-hrs ($p < 0.05$). There were no group x time interactions for any muscle function marker. Muscle soreness increased post-ECC ($p < 0.001$) and did not return to baseline. There were no group x time interactions for muscle soreness. Thigh circumference ($p = 0.456$) and HOMA-IR ($p = 0.396$) did not change post-ECC. IL-10 concentrations were below the valid detectable limits of the assay used, thus were not included in the analyses.

CONCLUSIONS: These data suggest that middle-aged men consuming 1.08 ± 0.02 g/kg/day PRO did not recover well from damaging exercise at +72-hrs and that pre-sleep protein, regardless of source, did not aid in muscle recovery when damaging exercise was performed in the morning.

PRACTICAL APPLICATION: Individuals should consume a sufficient bolus of high-quality protein in close proximity to their exercise session, whenever that session may be. Further, pre-sleep protein consumption can be utilized to consume enough protein throughout the day.

CHAPTER 1

INTRODUCTION

Starting as early as the fourth or fifth decade of life, muscle mass begins to decline (131). This age-related loss of muscle mass is termed sarcopenia. Sarcopenia is accompanied by decreased strength, which can lead to impairments in physical function, performance of daily activities, and other health concerns (15). According to the World Health Organization (WHO), the world's population 65 years of age or older was ~524 million in 2010 (8% of population) and is expected to increase to ~1.5 billion by 2050 (16% of population). With the aging population growing, elucidating methods to prevent sarcopenia and dynapenia (age-related strength loss) have massive societal and economic relevance. While resistance exercise (RE) is recommended for individuals of all ages (130) to increase muscular strength and power, improve body composition, and improve performance of activities of daily living, it may have particular importance for those of the aging population.

RE is a modality of training that is rarely disputed as the primary method for improving muscular strength and power and improving lean mass in persons of all ages. However, RE that is novel or of unaccustomed intensity can elicit muscle damage and delayed onset muscle soreness (90, 162). This damage is believed to be caused by disruptions to the contractile unit of the muscle, however, chemical processes from the resultant acute phase response may contribute as well (46, 115). Previous research has characterized the structural damage as z disc streaming, broadening, or total disruption when muscle biopsy samples were taken after damaging exercise (47). The same study found muscle soreness to persist for 18-72 hours following exercise and force loss during maximal voluntary contractions of the knee extensors both isometrically and at 90°/s, 180°/s, and 300°/s with decrements being greatest immediately after exercise, but still significantly lower than control at 90°/s and 180°/s at 3 days post-exercise and 300° /s at 6 days post-exercise. Many methods of reducing the negative effects of muscle damage, such as ice-water immersion, cryotherapy, and nutritional interventions (1, 44, 63, 70, 142, 152), have been previously investigated. Regarding nutritional interventions to mediate exercise induced muscle damage and potentiate recovery from damaging exercise, protein supplementation has gained considerable popularity anecdotally and in the literature.

Most work examining the effects of protein on muscle recovery has provided protein during the pre- or post-exercise timing window. Reidy et al. provided ~18 g of whey protein or ~19 g of casein, whey, and soy protein blend 1-hour post-exercise to healthy young participants. Anabolic signaling increased ($p < 0.05$) during the 2-hr recovery period and fractional synthetic rate (FSR) increased ($p < 0.05$) during the first 2-hrs and entire 4-hr recovery period for both protein groups (141). While muscle protein synthesis (MPS) can serve as a proxy for muscle reconditioning and recovery, it may not be an appropriate estimate of chronic muscular adaptation (172). Most research providing pre- and post-exercise protein supplementation has reported beneficial effects on chronic adaptations. Hulmi and colleagues reported that 30 g of post-exercise whey protein intake decreased fat mass ($p < 0.05$) and increased relative fat free mass to a greater extent ($p < 0.05$) than did isocaloric carbohydrate supplementation following 12 weeks of resistance training in untrained males (65). This is similar to earlier findings by Candow and colleagues where young men and women were provided with either whey protein + carbohydrate, soy protein + carbohydrate, or energy matched carbohydrate only, which served as the placebo (25). These authors reported both protein groups to have increased lean mass and 1 repetition maximum (1RM) strength for bench press and squat ($p < 0.05$) compared to placebo when consumed pre- and post-exercise and before sleep during a 6-week resistance training intervention. However, not all research has found protein supplementation beneficial for chronic muscular adaptation. Verdijk et al. reported no difference ($p > 0.05$) in 1RM strength, lean mass, or muscle fiber hypertrophy when 10 g of casein protein was provided pre- and post-exercise to elderly men during a 12-week resistance training intervention (171). It should be noted, however, that the 10 g per serving was likely insufficient to elicit a beneficial effect, especially in the aging population. Taken together, protein supplementation appears to have beneficial effects on MPS and therefore acute muscle reconditioning and recovery, as well as chronic adaptations to resistance exercise when consumed pre- and/or post-exercise.

A novel and emerging area of research regarding protein ingestion has been the pre-sleep feeding window. This timing of protein ingestion presents as an additional opportunity to consume nutrients, which may have beneficial effects on muscle protein synthesis, metabolism, and muscle recovery. In a proof of principle study, Groen and colleagues used an intragastric feeding

technique of intrinsically labeled casein protein or a placebo to determine protein digestion and absorption kinetics, as well as the resultant MPS response (53). These authors reported protein feeding increased MPS and net protein balance (11.8 ± 1.0 and 0.3 ± 0.1 μmol phenylalanine/kg/h for protein and placebo, respectively; $p < 0.05$). Further, they reported mixed-muscle FSR to be greater in protein compared to placebo (0.045 ± 0.002 and 0.029 ± 0.002 %/h, respectively; $p < 0.05$), indicating protein can be digested normally during the overnight period in healthy, older men. Similar results were found in healthy young males (142). Res et al. reported that 40 g of casein protein consumed pre-sleep following an acute bout of resistance exercise performed in the evening increased whole body MPS and net protein balance to a greater extent than did placebo (61 ± 5 vs. -11 ± 6 μmol/kg/7.5h for protein and placebo, respectively; $p < 0.01$) (142). Muscle FSR during the 7.5-hour overnight period was ~22% greater ($p = 0.05$) in protein compared to placebo. In addition to the acute MPS benefits, pre-sleep protein has been reported to have chronic benefits on muscular adaptation to resistance exercise. Snijders et al. reported pre-sleep ingestion of 27.5 g protein with 15 g carbohydrate increased muscular strength ($p < 0.001$), quadriceps muscle cross sectional area ($p < 0.05$), and type II muscle fiber size ($p < 0.05$) following 12 weeks of a progressive resistance training protocol compared to a noncaloric placebo (155).

Regarding metabolism, lipolysis, and next morning satiety, work from our lab demonstrates that pre-sleep protein ingestion has either beneficial or non-detrimental effects. Madzima et al. reported 30 g of pre-sleep casein to increase resting energy expenditure (REE) ($p < 0.05$) in active men (93). Ormsbee et al. reported improved next morning satiety ($p = 0.02$) in obese women when 30 g casein protein was consumed pre-sleep in comparison to whey protein or carbohydrate supplements (120). Kinsey and colleagues reported that consumption of 30 g pre-sleep casein protein did not impede overnight or morning fat metabolism in young obese men as determined by microdialysis techniques compared to a non-nutritive placebo (78). In summary, pre-sleep protein has been reported to improve the muscular reconditioning response to resistance exercise both acutely and chronically while improving (REE) and not inhibiting lipolysis or fat metabolism. While the benefits of protein consumption pre-and post-exercise have been widely examined, less is known about the effects of night-time consumption of protein on muscle recovery especially as it pertains to the source of protein consumed.

Therefore, while resistance exercise is recommended for all individuals to increase muscular strength and power, improve body composition, and improve performance of activities of daily living, this type of exercise can lead to delayed onset muscle soreness (DOMS), inflammation, decreased performance, and metabolic alterations if the exercise is novel or of unaccustomed intensity. Protein supplementation has been suggested to mediate these negative effects and enhance muscle recovery following damaging exercise. However, the work that has been done on the effects of pre-sleep protein ingestion has studied younger individuals and elderly persons which may not be translatable to middle-aged individuals. Little is known about the effects of protein supplementation on middle-aged individuals. Further, little is known about the effectiveness of dairy-based protein compared to plant-based protein sources, either in isolation or in combination, on younger or older populations. No studies have directly compared the effects of a dairy-based protein and plant-based protein in combination, such as a rice and pea protein combination on muscle recovery in younger or older populations. A novel area of research has been pre-sleep protein ingestion. This timing of protein ingestion represents an additional feeding window, which has shown benefit on MPS with possible benefit, but no harmful effects, on lipolysis. Despite evidence supporting benefits of pre-sleep protein, the type of protein consumed pre-sleep has not been investigated for its influence on muscle recovery.

1.1 Objective

The ***novel approach*** to this study is that despite evidence supporting benefits of pre-sleep protein, no studies identify if the type of protein consumed pre-sleep influences muscle recovery. Additionally, no studies have examined the effects of dairy-based or a combination of plant-based protein sources on muscle recovery. ***The purpose*** of this study was to compare the effectiveness of dairy-based or a combination of plant-based pre-sleep protein on muscle damage, metabolism, and performance following damaging resistance exercise (RE) in middle-aged men (40-64 years).

1.2 Specific Aims and Hypotheses

- **Aim 1 (Inflammation and Blood Markers):** To determine if 40 g of pre-sleep whey isolate (WI), whey hydrolysate (WH), or rice and pea combination protein (RP) reduces blood markers of muscle damage (CK) and inflammation (IL-6, IL-10, and CRP), as well as perceived soreness (visual analogue scale) and thigh circumference (Gulick tape) following eccentric exercise (ECC) induced muscle damage compared to placebo (PL) in middle-aged men
    - ***Hypothesis 1:*** Pre-sleep WI and WH will reduce blood markers of muscle damage and inflammation to a greater extent than RP and all treatments will be more effective than PL at 4, 6, 24, 48, and 72 hours post-ECC
    - ***Hypothesis 2:*** Pre-sleep WI and WH will reduce thigh circumference and perceived soreness to a greater extent than RP and all treatments will be more effective than PL at 24, 48, and 72 hours post-ECC
        - **Approach to test hypotheses:** Blood samples will be collected from an antecubital vein and analyzed via immunoassay for IL-6, IL-10, CK, CRP, and insulin. A visual analogue scale will be used to measure the perceived soreness in the lower limbs. Thigh circumference will be measured using a Gulick tape measure.

- **Aim 2 (Metabolic):** To determine if 40 g of pre-sleep whey isolate (WI), whey hydrolysate (WH), or rice and pea combination protein (RP) attenuates insulin resistance via HOMA-IR resulting from muscle damaging eccentric exercise (ECC) compared to placebo (PL) in middle-aged men.
    - ***Hypothesis 1:*** Pre-sleep WI and WH will attenuate insulin resistance resulting from muscle damaging exercise at 24, 48, and 72 hrs post-ECC to a greater extent than will RP and all treatments will be more effective than PL
        - ***Approach to test hypothesis:*** HOMA-IR will be used as a measure of insulin resistance.
- **Aim 3 (Performance):** To determine if 40 g of pre-sleep whey isolate (WI), whey hydrolysate (WH), or rice and pea combination protein (RP) potentiates the return of isometric and/or isokinetic maximal voluntary contraction (MVC) back to baseline

following damaging eccentric exercise (ECC) compared to placebo (PL) in middle-aged men.

- ***Hypothesis 1:*** Pre-sleep WI and WH will improve the return to baseline in muscular performance to a greater extent than will RP and all treatments will be more effective than PL at 24, 48, and 72 hrs post-ECC
    - ***Approach to test hypothesis:*** *MVC will be performed on a Biodex dynamometer to determine peak torque for isokinetic and isometric knee extension and flexion.*

1.3 Limitations

1) No direct measures of muscle damage such as muscle biopsies were collected during this study
2) Participants were men aged 40-64 years, thus, results may not be generalizable to other populations
3) Only one combination of plant-based protein supplements was used in this study
4) Only men were included in this study, thus results may not be applicable to women in a similar age range

1.4 Assumptions

1) The indirect measures of muscle damage were representative of actual muscle damage incurred from ECC
2) All participants performed all exercise testing at maximal effort
3) All participants ingested the supplement each day at the appropriate time
4) All participants recorded their habitual nutritional intake accurately

1.5 Delimitations

1) The anabolic endocrine responses to ageing and exercise, both in conjunction and separately, have been extensively studied. The implications of these hormones (i.e. testosterone and growth hormone) were unclear and, thus, were not being measured in this study.

2) All participants were eligible for the study based on pre-screening procedures, either by phone or by personal meeting.
3) This study included men between the ages of 40 and 64 years that were recreationally active, which was defined as engaging in physical activity on ≥ 2 days of the week for the past 6 months.

1.6 Terms and Abbreviations

Delayed Onset Muscle Soreness (DOMS) – Soreness in muscles following exercise that is novel or of unaccustomed intensity, usually becoming apparent within 24 hours following exercise

Eccentric Exercise (ECC) – Modality of RE by which the eccentric, or lengthening, component is emphasized or singularly performed

Excitation-Contraction (E-C) Coupling – The initiation of muscular contraction where a stimulus causes excitation at the neuromuscular junction, Ca^{2+} is released, and the formation of actomyosin is allowed to occur

Maximal Voluntary Contraction (MVC) – Greatest amount of torque produced by the participant

Placebo (PL) – Intervention group which consumed a noncaloric beverage 30 minutes pre-sleep

Resistance Exercise (RE) - Generally accepted modality of exercise for improving strength, power, body composition, and performance of activities of daily living where the musculature exerts a force against an opposing load

Rice and Pea Combination Supplement (RP) – Intervention group which consumed 40g of a combination of rice and pea protein 30 minutes pre-sleep

Visual Analogue Scale (VAS) – 100mm anchored scale that is labeled on either end with "no soreness" at 0mm and "extreme soreness" at 100mm used to assess perceived muscle soreness by having participant draw a vertical line on the scale to indicate their perceived muscle soreness level

Whey Protein Isolate Supplement (WI) – Intervention group which consumed 40g of whey protein 30 minutes pre-sleep

Whey Hydrolysate Protein Supplement (WH) – Intervention group which consumed 40g of whey hydrolysate protein 30 minutes pre-sleep

CHAPTER 2

REVIEW OF LITERATURE

2.1 Resistance Exercise

Resistance exercise (RE) is rarely disputed as the primary mode of exercise to increase strength, power, and lean mass. RE is now being recommended for individuals of all ages (130). The traditional mode of RE has concentric and eccentric components, however, other modalities do exist, such as eccentric specific exercise.

2.1.1 Eccentric Exercise

Eccentric exercise (ECC) is a mode of training where the muscle is lengthened while opposing a force. This is in contrast to concentric motions where the muscle shortens against a force or isometric contractions where the length of the muscle is unchanged (162). While traditional exercise has a component of ECC, individuals may engage in ECC specific exercise. ECC training is termed, "negatives" and is used by many weightlifters and strength athletes to increase muscle hypertrophy and performance due to the muscle regenerative response to eccentric exercise induced damage (76). Interestingly, ECC results in a lower metabolic cost and electrical stimulation of the muscle as read by electromyography (109). Further, early work by AV Hill showed energy liberation from the muscle is reduced during an eccentric contraction compared to that of isometric or shortening contractions, likely caused by a reduction in the rate of chemical processes within the muscle (58). This would help, at least in part, to explain the reduced metabolic cost of ECC. Despite this fact, ECC has been shown to elicit large amounts of muscle damage following a novel bout of exercise or following a bout of unaccustomed intensity (90, 162), and thus is often used to induce damage in research studies. ECC leads to delayed onset of muscle soreness (DOMS), which is characterized by pain and/or tenderness following unaccustomed exercise and that has a delayed onset. This muscle soreness is believed to be caused by disruptions to the contractile proteins of the muscle tissue, however; a combination of myofibrillar disruption and chemical processes may be involved (46, 115). It has been suggested that ECC can cause metabolic alterations (3, 80, 119, 162), performance decrements (34, 102, 140, 144), and Ca^{2+} flux alterations (139) such that Ca^{2+} fluxes into the sarcoplasm (150). This

increased intracellular calcium concentration is presumably a result of increased sarcolemma permeability which disturbs calcium homeostasis within the muscle and can activate calpains, furthering damage (12, 36). Glucose disposal rates during euglycemic-hyperinsulemic clamps were reduced for ECC (3.47 ± 0.51 mg/kg/min) compared to concentric exercise (5.55 ± 0.98 mg/kg/min) or control (5.48 ± 1.0 mg/kg/min) at 48 hours (80). Similarly, using a crossover study design with ECC downhill treadmill running or non-exercise control and hyperinsulinemic-euglycemic clamp, Del Aguila et al. reported reduced insulin stimulated IRS1 tyrosine phosphorylation, PI3K activity, and Akt serine phosphorylation in ECC compared to control. These molecular alterations were reported in combination with glucose disposal rates that were 19% lower following the ECC versus the non-exercise control trial (3). Crameri et al. used 100 and 110 maximal eccentric quadriceps contractions at 30°/s and 180°/s, respectively, at a knee angle of 70° to induce damage in sedentary men. This protocol reduced maximal voluntary contraction (MVC) of the quadriceps muscle by 16%, 25%, and 8% at 4, 24, and 96 hours post ECC, respectively. They also noted increased muscle soreness both at rest using a blunt probe applied at a standardized pressure and during maximal voluntary contractions at 24 and 96 hours as indicated by visual analogue scale (34). This decrement in force production and strength has been found in other studies for women (140, 144) and trained men (102). Previous research has used various ECC protocols to induce muscle damage such as downhill running, eccentric cycling, drop jumps, and opposing a weight or level using isokinetic dynamometers or free weights (63, 79, 101, 102, 109).

While ECC is well established to induce muscle damage, the exact mechanisms by which ECC causes muscle damage and soreness have yet to be elucidated. The initial event of muscle damage is most typically described as being mechanical in nature (109), causing disruption to the structural components of the muscle cell due to the force exerted by the tissue. While the mechanical model of injury is most widely accepted, it has been proposed that injury is metabolic in nature where exhaustion from exercise induces metabolic deficiency within the muscle and the disrupted metabolic state increases the vulnerability of the cell to damage (139). Pursuant to mechanical disruption is a secondary response including an inflammatory response, flux of intracellular proteins to the extracellular space and blood, performance decrement, and

metabolic alterations such as reduced insulin sensitivity, glycogen repletion, and alterations in Ca^{2+} kinetics.

2.1.1.1 Muscle Structure and Function

The contractile unit within the muscle fiber is termed the sarcomere. Sarcomeres within the muscle fiber are arranged both in series and in parallel. Sarcomeres of muscle fibers are characterized by two parallel Z-disks with the M-line anchored to intermediate filaments midway between the Z-disks. Actin filaments are anchored to the Z-disk by nebulin with myosin filaments arranged in an interdigitated fashion. Titin (Connectin) filaments link myosin to the actin and/or Z-disk and possibly to other elastic components near the Z-disk. It is postulated that the elastic proteins titin and nebulin play a role in maintaining Z-disk integrity and resting tension as well as stretch resistance (45, 98, 167). When the muscle is stretched such that the actin and myosin no longer overlap, sarcomeres are "popped." This is non-uniform during ECC and sarcomeres "pop" in order of those with weakest tensile strength first (106). Not all sarcomere "popping" causes damage. The elastic components help maintain structure to return the sarcomere to normal resting tension and alignment. However, not all sarcomeres return to normal and, thus, sustain damage. When participants performed damaging eccentric exercise on a dynamometer, titin and nebulin concentrations were reduced by 30% and 15%, respectively 24 hours post exercise (167). This indicates that the myofibrillar proteins were damaged and thus, degraded and removed via proteolytic action. Considering titin and nebulin anchor myosin and actin to the Z-disk, respectively, damage to these proteins, at least in part, contributes to the sarcolemmal disruptions following ECC. Of interest, previous research has indicated that older individuals, both male and female, are susceptible to greater levels of ultrastructural damage to the same ECC compared to younger individuals (96, 146).

Functionally, muscles contract via excitation-contraction (E-C) coupling and the sliding filament theory. For a more in depth description, the reader is referred to a review of the topic (66, 69). Briefly, E-C coupling is the process by which a stimulus causes the contractile units of the muscle fibers to produce muscular contraction. When an action potential, traveling down the axon of a motor neuron, reaches the neuromuscular junction, Ca^{2+} enters the depolarized pre-synaptic terminal which triggers acetylcholine release into the synaptic cleft via vesicular

exocytosis. Acetylcholine binds and opens acetylcholine receptor proteins in the post-synaptic membrane, depolarizing the myofiber and initiating an action potential. This causes Ca^{2+} release from the sarcoplasmic reticulum into the cytoplasm of the muscle fiber. Ca^{2+} binds troponin molecules on thin filaments, causing conformational changes that exposes myosin binding sites on actin due to movement of tropomyosin. Thus, myosin heads on thick filaments are able to bind actin to form actomyosin cross-bridges, which results in a marked rate of ATP hydrolysis (66). Each cross-bridge can undergo a "power stroke," the sum of which results in force production and muscle shortening if the force produced overcomes the load that the muscle is subjected to. According to the sliding filament theory, sarcomere shortening occurs due to the combined action of cycling cross-bridges which use the energy from ATP hydrolysis for myosin to "pull" on actin, thus shortening the sarcomere (67, 68). Neither thick nor thin myofilaments changes length, but rather can "slide" against each other, resulting in muscle shortening. Binding of ATP to the myosin causes dissociation of the actomyosin. This ATP is hydrolyzed into ADP + Pi, creating a "charged" myosin. If Ca^{2+} is still present, the myosin head will, again, bind the actin, continuing contraction. This will continue until the stimulus input is no longer present or sufficient to elevate cytoplasmic Ca^{2+} in the muscle fiber (69).

When a muscle contracts, heat is produced and liberated from the fiber both from the contraction and from the mechanical work done. Extensive work by A.V. Hill on the thermodynamic properties of muscle has been done. Hill found the amount of energy liberated is determined by factors such as type of contraction, load, velocity, muscle length, cross sectional area, contraction length, and temperature. Hill also found that the thermodynamics of muscle contraction mimicked that of the mechanics of muscle contraction, thus heat could be used to predict the force-velocity relationship. To this effect, Hill described this force-velocity curve with equation:

$$(P+a)\,(V+b) = c$$

In this equation P is load, V is velocity and *a, b, and c* are constants. It was later determined that *a* is not, in fact, a constant, but determined by shortening velocity and load (59).

Regarding type of contraction, muscle shortening produces the greatest amount of heat, followed by isometric contractions, and lengthening contractions producing the least heat as work is being done onto the muscle fiber. Hill proposed that during an isometric contraction, the rate of heat production equals tension. Should the load be lowered such that the muscle shortens,

more work is done, hence greater heat production. Conversely, if the load is increased such that the muscle is lengthened, the muscle does negative work. That is to say, the load exerts work upon the muscle, thus heat production is lower (58). Hill goes on to describe the relationship of load and velocity during shortening contractions. Briefly, load is inversely related to velocity. As load increases, velocity decreases. When load is decreased, velocity increases. While the work done over a given distance is greater with a larger load, total heat for shortening is unchanged (58). This force-velocity relationship is based upon the cross bridging of the actomyosin contractile proteins that occurs during excitation-contraction coupling (151), which has been briefly described above. Furthermore, this relationship can be used to describe maximal power output for a given velocity and load.

2.1.1.2 Mechanical Disruption

The mechanical model suggests that unaccustomed exercise, especially ECC, results in disruption to the sarcomere (47, 109). Interestingly, most of this damage is localized to type II fiber types (47). It has also been shown that males and females have different responses to EIMD. Following a downhill running protocol, female rats showed a slower and less robust change in myofiber swelling, necrosis, and disruption to sarcomeric proteins than did male rats (83) Fridén and colleagues reported that eccentric cycling in college aged males produced myofibrillar z-disc streaming, broadening, and total disruption, detected by direct measures of muscle biopsy. Further, damaged areas displayed absence of mitochondria and transverse sections of the A-bands showed some disturbance or absence of thick myofilament. Indirect measures in the same study indicated increased muscle soreness 18 – 72 hours after the exercise bout. Decreased maximal knee extensors force was found isometrically and at 90°/s, 180°/s, and 300°/s with decrements being greatest immediately after exercise, but still significantly lower than control at 90°/s and 180°/s at 3 days post-exercise and 300° /s at 6 days post-exercise (47). Newham et al. used 20 minutes of either concentric or eccentric stepping. Muscle biopsies taken pre-exercise, immediately post-exercise, and between 24- and 48-hrs post-exercise indicated that only the eccentric protocol elicited damage and that damage was greatest between 24- and 48-hrs post exercise. In samples taken between 24- and 48-hrs, 6% of fibers showed focal damage, 23% showed extensive damage, and 28% showed very extensive damage compared to immediately

post-exercise, which showed 16% having focal damage, 16% having extensive damage, and 8% having very extensive damage (109).

2.1.1.3 Force Loss

Immediately following an ECC exercise bout, a reduction in force production is noticed (18, 29, 150, 160), representing damage to the excitation-contraction (E-C) coupling mechanism (138). Force production decrements are usually less pronounced and return more quickly to baseline following concentric or isometric exercise compared to eccentric exercise (27, 56, 150). It appears that this loss of force is only partly attributed to structural damage but other factors are likely contributing (56), perhaps metabolic or neural in nature. However, after a damaging bout of eccentric exercise, force production is reduced for a longer duration, on the order of days (18, 160). Some researchers have noted that force decrements still exist 11 days following damaging exercise (29). When a total of 70 maximal voluntary contractions of the elbow flexors each lasting 3 seconds in duration was performed in female physiotherapy students (21.4 ± 3.3 years old), isometric force was reported to be reduced ($p < 0.01$) by 20% 11 days post-damaging exercise (29). It has been shown that glycogen repletion within the muscle tissue is inhibited after muscle damage, which may impair the ability of the fiber to undergo repair (119). ECC induced force loss appears to have a modality or intensity component. It has also been shown that force loss is a function of the length of the tissue at the onset of the ECC. Greater starting muscle lengths induced more appreciable reductions in force than did shorter starting muscle lengths. A review of the topic has suggested that an association between the extent of force generation loss and myofibrillar damage, fiber necrosis, and inflammation response exists such that greater losses of force represents greater damage (123). While no exact definition or criterion for muscle damage currently exists, these authors go on to suggest the term “mild exercise induced damage” for force loss of under 20% and/or return to baseline is within 48-hrs while “moderate” or “severe” exercise-induced damage produces greater than 20% force decrements persisting for greater than two days. Interestingly, nutritional interventions have been reported to have mediated the detrimental effects of exercise induced muscle damage. Cooke and colleagues showed that protein supplementation in healthy, untrained males was able to attenuate the strength reduction following 4 sets of 10 eccentric leg press, leg extension, and leg flexion exercises. Isometric knee extension strength was greater in the whey protein plus carbohydrate

group than carbohydrate group at 3 days ($p < 0.05$) and 7 days ($p < 0.01$) post-exercise (32). Similar results have been found with leucine supplementation. Squat isometric peak force was reduced in both leucine and placebo groups, however, reductions in force were attenuated ($p = 0.04$) at all time points compared to placebo (79).

2.1.1.4 Muscle Soreness

Muscle soreness is often most noticeable in the day or days following damaging exercise, thus given the term delayed onset of muscle soreness. The soreness usually peaks at 48 to 72-hrs but may remain for multiple days (63, 116). This soreness can happen when exercise is novel, such as with sedentary individuals, or of an unaccustomed intensity, such as when increasing training volume in trained individuals. Muscle soreness is often examined when investigating muscle damage or effectiveness of various treatments on muscle damage (173). When 26 females were subjected to 70 maximal voluntary contractions of the elbow flexors, muscle soreness was elevated ($p < 0.01$) at 24-hrs, peaked at 72-hrs (29). This work agrees with the findings from other research using various damaging exercise protocols (18, 52, 72, 79, 100). As seen with force loss, protein supplementation has been reported to mediate muscle soreness (52, 63, 70, 153).

It has been proposed that muscle soreness weakly correlates with structural damage (115). Others have suggested soreness may be caused, at least in part, by lysosomal enzyme activity (46) or reactive oxygen species (165). Unfortunately, high variability in ECC protocols from previous research makes comparisons of soreness difficult between studies. It has also been reported that the method of assessing muscle soreness alters the soreness outcome. For example, when muscle soreness was assessed during flexion, soreness was lower ($p < 0.01$) than when assessed during palpation or extension of the same muscle group following 12, 24, or 60 maximal eccentric actions of the elbow flexors, with extension and palpation being similar in extent of soreness (115). These authors went on to suggest muscle damage and loss of function may be present in the absence of soreness, thus soreness should not be used in isolation to assess the magnitude of exercise-induced muscle damage, but in conjunction with other indicators. The physiological mechanisms or significance of DOMS have yet to be elucidated.

2.1.1.5 Blood Markers of Muscle Damage

Exercise which elicits muscle damage has been associated with myofiber protein leakage and a local and systemic inflammatory response (91). Creatine Kinase (CK), Lactate Dehydrogenase (LDH), C-Reactive Protein (CRP), and Myoglobin are often measured as indicators of muscle damage (91). Myosin heavy chain (MHC) analysis has also gained popularity as an indicator of damage. Due to the inflammatory response, cytokines, in particularly interleukin-6 (IL-6), have been used frequently to assess muscle damage. Cytokines play an integral role in the inflammatory response and are believed to initiate the acute phase response (139). These cytokines are released at the site of injury during the local response and trigger the systemic acute phase response (described in more detail in section 2.1.2). They can be characterized as pro- or anti- inflammatory and regulate the immune response to trauma and tissue damage (121). While IL-6 is considered a pro-inflammatory cytokine and important in the muscle adaptive response (158), IL-10 inhibits the release of TNF-α and IL-1β, thus inhibiting inflammation. Both IL-6 and IL-10 concentrations have been reported to be increased following damaging exercise (91, 121). Following 300 eccentric repetitions on an isokinetic dynamometer, IL-6 was elevated ($p < 0.001$) immediately following exercise and peaked 6-hrs post exercise. At 24-hrs post-exercise, IL-6 was still significantly elevated ($p < 0.05$) from pre-exercise concentration (91). This is similar to the findings of Ostrowski and colleagues, who showed IL-6 and IL-10 (An anti-inflammatory cytokine) concentrations to increase 128-fold and 27-fold, respectively, immediately following participation in a marathon (121).

Creatine kinase is an enzyme which catalyzes the reversible transfer of phosphate from adenosine to creatine, which allows ATP production from creatine-phosphate and ADP. Cytosolic CK is dimeric and is composed of either B subunits (brain form) or M subunits (muscle form). The M subunits, or muscle form subunits, have multiple isoenzymes (CKBB, CKMB, and CKMM), with CKMM being most predominant in skeletal muscle tissue (158). In ECC naïve males, Nosaka and colleagues used 24 maximal eccentric actions of the elbow flexors to elicit damage. CK concentrations increased ($p < 0.05$) from days 1 to 5 post-exercise, with the peak being at day 4 (797 ± 288, 6283 ±2387, 11,599 ± 2923, 11,932 ± 1731, and 9262 ± 1302 $IU \cdot l^{-1}$ for days 1 through 5, respectively). Interestingly, this same study found no increases in IL-6, myoglobin, or CRP, but did find increases ($p < 0.05$) in LDH at day 2 through 5 post-

exercise (113). CK and Myoglobin concentrations were increased ($p < 0.05$) at 24-hrs post-exercise when 100 depth jumps from 60cm were performed, but were not significantly elevated at any other time point (79). It has been previously suggested that the variability found in CK concentrations following ECC is related to the variability in muscle damage (114) thus, it could be that the depth jump protocol elicited less damage than did the maximal elbow flexor protocol. This could be due to the intensity at which the exercises were performed (i.e. maximal vs. submaximal). Using an eccentric cycling protocol, Manfredi et al. elicited muscle damage in young and older men to determine the effects of aging on muscle damage from ECC. CK concentrations were elevated at days 5 ($p < 0.03$) and 8 ($p < 0.02$), and were still elevated at 10 days post-ECC ($p < 0.03$) with no differences in age groups (96). It appears that younger and older males have similar biochemical responses to damaging ECC. However, male and females seem to have different responses. Hicks et al. showed greater serum CK concentrations ($p<0.05$) following 6 sets of 12 repetitions of eccentric MVC of the quadriceps in males compared to females at 1, 48, 96, and 168-hrs post-exercise (57).

2.1.2 Immune Response

Subsequent to the initial mechanical damage from ECC, an immune response is triggered to repair the damaged tissue. The local response to damaged tissue is the production of cytokines, increased blood flow, and vascular permeability (91, 121). These cytokines cause an influx of systemic immune cells to the localized site of injury. The systemic response, otherwise referred to as the acute phase response, involves immune cells such as lymphocytes, neutrophils, and monocytes being mobilized to the damaged tissue to begin the repair process. These cells play a role in the phagocytosis of cellular debris and necrotic tissue. While they have a role in debris clearance, they may contribute to muscle damage, perhaps through cytotoxic and oxygen radical mediated processes (165). It has been proposed that cytokines may be contraction mediated and, thus, not specific to eccentric exercise or muscle damage (145). Rather, it appears that cytokines are produced by the muscle fiber, termed “myokines,” and act as growth factors to aid in regulation of muscle growth and metabolism (16, 111). Expression of IL-15 mRNA (111) and leukemia inhibitory factor mRNA (16) were upregulated following concentric exercise. This supports the notion that cytokines, or myokines, are contraction mediated. Thus, while they

contribute to muscle reconditioning following damaging ECC, they are not unique to this modality of training.

Neutrophil infiltration begins within one hour, peaks at 6-hrs (91, 94), and may remain elevated for hours to days after exercise (164). Like neutrophils, macrophages are phagocytic, aid in debris clearance of damaged tissue, and can secrete cytotoxins. Macrophages are differentiated from monocytes and play a role in cytokine production to mediate inflammatory responses, and promote repair (39). Macrophages have two different phenotypes: M1 and M2. M1 macrophages are coined as being pro-inflammatory, secreting IL-6, TNFα, and IL1β following damage while M2 macrophages are considered anti-inflammatory, secreting IL10 later in the repair process (180). Therefore, it seems that cytokine and immune cells work in conjunction during the acute phase response to repair the damaged muscle tissue resulting from exercise.

2.1.3 Repeated Bout Effect

It has been well documented that ECC elicits muscle damage. An interesting phenomenon, known as the repeated bout effect, has been noticed with subsequent ECC sessions. While the initial bout elicits a large amount of damage, repeated bouts elicit much less damage (116, 117). Interestingly, it seems that initial bouts of concentric exercise also elicit a repeated bout effect for ECC exercise (76) and that the repeated bout effect is not exercise specific, such that varied exercise bouts can lead to protection in a second bout of exercise (183). The mechanisms by which this protective mechanism happens has not been fully elucidated, however, some suggest it is due to removal of fibers of weaker tensile strength, an increased ability to repair damaged tissue, or increased connective tissue content (18). Further, a review of the topic has suggested neural adaptations by which motor unit activation patterns are altered, mechanical adaptations by which muscle stiffness alterations play a role, and cellular adaptations whereby altered inflammatory responses, longitudinal addition of sarcomeres, and altered excitation-contraction coupling influences the subsequent bout of ECC (For full review see (100). Nosaka and colleagues had men perform 24 maximal eccentric contractions of the elbow flexors separated by either 6, 9, or 12 months to determine the duration of the repeated bout effect. Maximal isometric force dropped to 47 ± 1.5 % after the first bout, with no difference in the initial force reduction following the second bout in either group. However, recovery of force was improved ($p < 0.05$)

in the 6-month and 9-month groups, but not the 12-month group. Muscle soreness was reduced ($p < 0.01$) for the 6-month group after the second bout, but not in the 9-month or 12-month groups. Similar to muscle soreness, CK concentrations were significantly increased after the first bout (19,403 ± 1,677, 20,590 ± 2,184, and 16,131 ± 2,252 $IU \cdot L^{-1}$ for 6, 9, and 12 months, respectively; $p < 0.01$) but were attenuated ($p < 0.01$) in the 6-month group only (116). It should be noted that the authors reported large variability of plasma CK concentrations amongst the participants. This study suggests that the repeated effect may last longer than previously thought, with protective adaptation possibly lasting up to 6 months. It should also be noted that the protective effects seem to have an intensity and source specific adaptation such that higher intensities need be trained in the initial bout to elicit protection of subsequent bouts and only muscles involved in the ECC are subject to the repeated bout effect (100). This intensity component of the initial bout has been previously reported. Howatson et al. found that while an initial bout of exercise using 45 maximal eccentric contractions elicited more muscle soreness ($p < 0.001$) and isometric force loss ($p < 0.001$) in the first bout than 10 maximal eccentric contraction, the protective effects were similar between the groups for the repeated bout (64). It would seem that intensity of the initial bout, but not volume, is the primary variable in determining the protective effects of an initial bout of ECC on subsequent bouts.

2.1.4 Measuring Muscle Damage

There has been some debate as to the most appropriate techniques to measure muscle damage. Traditionally, muscle biopsy and histology have been used as direct measures of muscle damage. However, some research is indicating this may not be optimal (94). In a clever study design, Malm et al. performed multiple muscle biopsies and blood draws on individuals at select time points following either eccentric cycling or no exercise control. While most immunological markers were elevated, no differences were reported between groups for muscle or blood neutrophils or macrophages, satellite cell activation, or IL-1β. Furthermore, this study noted large variation between individuals as determined by large confidence intervals (94). It appears that muscle biopsy and ECC induced muscle damage elicit similar immunological changes, thus, complicating the results of studies which have used this method. In addition to the findings of Malm and colleagues, Warren et al. raised the question of a biopsy sample being representative

of the muscle tissue as a whole (173). While this point is valid, the muscle biopsy is still likely the most advanced method currently available for muscle analysis.

In a review of measurement methods used for ECC-induced muscle damage and their efficacy, Warren et al. reported the most common measurement techniques in humans to be muscle soreness (73%), blood protein biomarkers (52%), and MVC (50%) with others using histology, range of motion, or other indices (173). These authors suggested that force related measurements, such as isometric and isokinetic MVC, should be included in analysis. Until a definition is clearly stated for muscle damage, a combination of variables is likely the best approach to determining the magnitude of ECC-induced muscle damage.

2.2 Muscle Recovery

2.2.1 Protein Intake

Protein ingestion is accepted as a key macronutrient in the muscle reconditioning response and for stimulating muscle protein synthesis (MPS) in all age groups. Previous research has challenged the Recommended Daily Allowance (RDA) of 0.8 g/kg of protein in favor of increased doses for athletes, those wishing to improve body composition, and older adults. Research suggests these dosages should be higher in healthy individuals without health consequence, particularly in regard to renal function (26). While this topic is beyond the scope of this review of literature, the interested reader is directed to reviews of the topic (11, 132–136).

2.2.1.1 Protein Dose

Previous work has been completed to determine the optimal per serving dose of protein ingestion. In young, college-aged, resistance trained people, 20 g of protein has been shown to maximally stimulate muscle protein synthesis (MPS) in the resting and exercised postprandial state (176). Fractional synthetic rate (FSR) increased ($p < 0.05$) by ~49% and ~56% for 20 g and 40 g, respectively, compared to 0 g. Additionally, compared to 10g, FSR was increased ($p < 0.05$) by ~22% and ~28 for 20 g and 40 g, respectively. No differences were seen in MPS between 0 g and 10 g ($p > 0.05$). Further, slight, but not significant ($p > 0.05$), increases in FSR in the rested and exercised state for 40 g compared to 20 g of whey were found. However, amino

acid oxidation and urea production area under the curve was elevated, indicating a fate other than muscle protein synthesis (176). The results of this study indicate that 20 g of high-quality protein is sufficient to induce maximal MPS in young people. Intakes in excess of this amount contribute minimally to the muscle reconditioning response when the protein is of sufficient quality. However, this study utilized unilateral leg resistance training only. When whole-body resistance training was performed, 40 g whey protein stimulated MPS to a greater extent than did 20 g ($p = 0.005$) in young, resistance trained men (92). Thus, the dose-response is dependent on the amount of muscle mass stimulated during training. Individuals seeking to maximize MPS should ingest at least 20 g of high-quality protein per serve with greater doses being required when whole-body training is performed. Of late, research has examined the necessary dose of protein to maximize MPS in aging and older individuals.

Work investigating optimal per serving dose is somewhat equivocal in older adults and further work need be done to elucidate the dose response to protein ingestion in aging individuals. While 20 g of protein was sufficient for younger individuals, older persons require a greater dose per serving. Older individuals display an "anabolic resistance" and a greater protein requirement is needed to maximally stimulate MPS (129, 181, 182). When either 10 g, 20 g, or 35 g of whey protein was given to healthy, older adults, 35 g whey protein led to greater aminoacidemia and amino acid absorption. This dose was also more effective at stimulating MPS than 10 g or 20 g in the resting state. FSR increased ($p < 0.05$) from the basal state by 44 ± 16% for 35 g while neither 10 g nor 20 g was sufficient to elevate MPS above basal rates (129). Yang and colleagues used an elegant model to determine FSR using constant infusion isotopic tracers with unilateral resistance exercise whereby the non-exercise leg served as the fed only control. Generally healthy, lightly to moderately active older adults were given either 0 g, 10 g, 20 g, or 40 g of whey protein isolate following the unilateral resistance exercise bout. Interestingly, 20 g of whey protein was sufficient at stimulating MPS in the non-exercised state ($p < 0.05$). The exercised state increased FSR for all whey doses compared to the non-exercised state ($p <0.05$). Myofibrillar FSR was elevated ($p < 0.01$) in the exercised state with both 20 g and 40 g compared to 0 g or 10 g. However, 40 g stimulated myofibril FSR to a greater extent (~32%, $p < 0.02$) compared to 20 g in the exercised state (181). This would suggest that the combination of protein intake and resistance exercise is a potent stimulator of MPS. Further, resistance exercise

seems to exaggerate the effects of nutritional stimulation of MPS alone. Work by the same group found that neither 20 g nor 40 g of soy protein was sufficient to stimulate MPS at rest and only 40 g of soy protein stimulated MPS post-exercise ($p < 0.05$) in a similar population. Both resting and exercised MPS was greater ($p < 0.001$) with whey compared to soy for the respective doses. Similar to the findings of other researchers 40 g of whey stimulated FSR to a greater extent ($p < 0.05$) compared to 20 g whey protein post-exercise in older men (182). This work indicates a dose- and source-dependent nature of protein ingestion. Taken together, aging individuals require greater per serving doses of protein to maximally stimulate MPS and the muscle reconditioning response both at rest and post-exercise. Additionally, this response is mediated by factors such as dose, source, and exercise state. It should also be noted that all of these studies used whole proteins, rather than essential amino acid (EAA) or branched chain amino acid (BCAA) supplementation.

2.2.1.2 Essential Amino Acids and Branched Chain Amino Acids

Proteins are made up of chains of their component parts, amino acids. The body synthesizes some of the amino acids. These are called non-essential amino acids and have no influence on net nitrogen balance (105). However, the body is unable to synthesis some amino acids, termed essential amino acids (EAA), and must be consumed through the diet. Of the EAA, the branched-chain amino acids (BCAA) comprised of leucine, isoleucine, and valine, have gained popularity in the sports supplementation and nutritional industries. As discussed in section 2.2.1.3, leucine has unique properties in its ability to stimulate anabolic processes such as the mTORC1 pathway. As such, it is no surprise that supplement companies and athletes have tried to take advantage of this anabolic amino acid via BCAA or EAA supplementation.

In a study examining the effects of a nitrogen balanced EAA supplement either with or without leucine, 8 healthy, recreationally trained women were provided a total of 260 mg EAA/kg body weight in small boluses (150ml solution throughout the experiment) during a resistance exercise bout and 180-minute recovery period, with total leucine intake being 45 mg/kg body weight in the EAA with leucine group. Both mTORC1 and p70S6k phosphorylation were increased with both supplements post exercise. However, mTORC1 phosphorylation was increased to a greater extent at 1 hour of recovery (120% and 46% for EAA with leucine and EAA without leucine,

respectively; $p < 0.05$). Additionally, the downstream protein p70s6k phosphorylation was enhanced by 59-fold compared to 8-fold ($p < 0.05$) for EAA and EAA without leucine, respectively. The mRNA levels of negative modulators of mTORC1 such as regulated in development and DNA damage responses 1 (REDD1), muscle atrophy F-box (MAFbx), and muscle ring finger-1 (MuRF-1) were reduced ($p > 0.05$) following supplement ingestion, with no differences between groups (105). This research suggests leucine is required to maximally stimulate the anabolic mTORC1 pathway following resistance exercise but has little to no effect on muscle protein breakdown. In a follow up study seeking to determine if leucine mediated activation of mTORC1 is potentiated by BCAA or EAA, 8 resistance trained men were provided in a randomized and counter-balanced order either placebo, leucine only, BCAA, or EAA. Immediately post-exercise, all supplements increased mTOR phosphorylation, including placebo. While mTOR phosphorylation was still elevated at 90 minutes of recovery with placebo, it was elevated to a greater extent with the other treatments (37% leucine only, 57% BCAA, and 71% EAA). At 180 minutes of recovery phosphorylation of mTOR was still elevated above baseline but to a greater extent with BCAA and EAA ($p < 0.05$). S6K1 activity saw similar findings to mTOR phosphorylation, except EAA elevated activity to a greater extent ($p < 0.05$) than all other trials at 90 minutes of recovery (104). This study agreed with previous findings that leucine is necessary for stimulation of the anabolic pathway of mTORC1 and its downstream signaling proteins, however, these results are enhanced with co-ingestion of BCAA and EAA. However, it has been suggested that a disconnect exists between activation of mTORC1 signaling and the protein synthetic response (71). Thus, Jackman et al. sought to determine myofibrillar synthetic response to BCAA ingestion without other EAA, intact proteins, or macronutrients following exercise. In a well-controlled study, 5.6g of BCAA were ingested by 10 healthy, resistance trained males following a bout of resistance training. Anabolic signaling was increased ($p < 0.05$) at 1 hour of recovery where phosphorylation of PRAS40 increased ~12-fold and S6K1 phosphorylation increased ~6-fold compared to placebo. Myofibrillar FSR was increased by ~22% ($p = 0.012$) in BCAA compared to placebo. The primary finding of this study was that while BCAA ingestion following exercise is able to stimulate MPS, stimulation is submaximal. This was likely due to an increased ability for EAA utilization following resistance exercise. Thus, the limiting factor seems to be substrate availability of EAA to support maximal MPS. The secondary finding of this study is that it

appears increased anabolic signaling and elevated MPS are associated. Taken together, it seems leucine is required to stimulate anabolic signaling and MPS. However, without adequate EAA availability, MPS will be submaximal and induce limited muscle reconditioning in response to resistance exercise. It has been suggested, though, that EAA consumption stimulates MPS to a greater extent when taken in combination with carbohydrate pre- rather than post-exercise, likely due to enhanced amino acid delivery to muscle tissue (166). However, the combination of carbohydrate makes direct comparisons of findings difficult. Generalizations cannot be made at rest, however, following resistance exercise a sufficient dose of leucine, BCAA, and EAA should be ingested to maximally stimulate the synthetic response and muscle reconditioning response in young people. All of these studies were in young, resistance trained individuals. Given the body of literature stating an anabolic resistance with age, it seems likely that results would be similar to that of the presented studies, and that doses need be in excess of those suggested in the studies presented. One study did attempt to look at the effects of chronic consumption of a leucine rich (3.2 g) BCAA supplement or placebo on muscle recovery following 12 weeks of a resistance training protocol in an aging population (55-75 years old). While the beverage attenuated the decline in muscular strength at the acute phase of recovery (0-3 hrs post-ECC), no benefits were found for MVC during the regeneration phase (24-72 hrs post-ECC), muscle soreness, or biomarkers of muscle damage (143). It is possible that this dose was not sufficient for the population due to the noted anabolic resistance. Thus, future work need be done to examine the effects on aging populations.

Though the acute muscle synthetic response to exercise seems to be blunted with consumption of only BCAA, others have noted benefits on muscle recovery following exercise. In regard to muscle recovery, studies have found benefit to consumption of BCAA supplementation on muscle soreness, return of muscle function and performance, biomarkers of muscle damage (CK, LDH, Myoglobin), and ratings of perceived exertion (14, 33, 63, 70, 99, 153). This is similar to findings consuming protein supplements such as whey (32) or milk concentrate (38). While some research has found no benefit (4), most research supports the use of BCAA and EAA protein containing protein supplements for muscle recovery. While leucine and BCAA supplements do exist, consuming intact protein sources or high-quality protein supplements may be more optimal than leucine or BCAA, alone.

2.2.1.3 Dairy- vs. Plant-Based Protein

Leucine is often considered an important EAA for protein synthesis in part due its ability to trigger MPS. Leucine has been documented as a signaling molecule which regulates satiety, insulin secretion, and skeletal muscle anabolism (177). Leucine is unique amongst the BCAAs for its ability to stimulate MPS through a mechanistic target of rapamycin (mTOR) pathway (5), which is a potent stimulator of growth. Recent investigations propose that this stimulation is accomplished through a leucine-binding protein Sestrin 2 via Sestrin2-GATOR2 interaction (177). Thus, stimulating MPS requires a bolus of protein to be of either sufficient quality and/or dose to contain appropriate leucine content.

Because leucine is unique amongst the EAAs to stimulate MPS, it is perhaps the most important amino acid in accrual of lean mass. Aging is often associated with muscle degradation, ultimately leading to sarcopenia and reduced function. In the aging populations, where muscle is degraded and sarcopenia may arise, increased leucine content is needed to maintain or stimulate muscle growth (74). Using a primed, constant infusion of labeled phenylalanine in conjunction with muscle biopsy and arteriovenous blood sampling, FSR and net nitrogen balance was determined in young and elderly individuals with ingestion of an EAA beverage containing 26% leucine or enriched with 41% leucine. As would be expected, the 41% leucine beverage elevated blood leucine concentrations above basal levels to a greater extent and for a longer duration (105 min. vs. 120 min. for the elderly and 90 min. and 150 min. for the young groups for 26% and 41% leucine, respectively) than did the 26% leucine beverage ($p < 0.05$). Both the 26% and 41% leucine beverages increased FSR in the young group ($p < 0.05$). Interestingly, only the 41% leucine beverage increased FSR in the elderly group (74). While the need for older individuals to consume greater per serving protein doses has already been discussed, these results suggests that greater leucine content, rather than dose, may be critical for stimulating postprandial MPS and maintenance or accrual of lean mass. Due to this, leucine content of a protein source is critical when discussing whole food or supplement sources. Various sources of protein, be it whole food or supplements, contain differing amounts of leucine. Table 1 lists the leucine amino acid reference ratio (AARR) for relevant protein sources to this review of literature (148). Dairy-based proteins contain higher concentrations of leucine compared to plant-based proteins. Soy proteins do have a relatively high leucine concentration amongst other plant-based protein

counterparts; however, this concentration is still lower than dairy-based proteins. Practically, aging individuals would be able to consume smaller portions of dairy-based proteins compared to plant-based proteins to stimulate MPS, improve lean mass, and mediate age-related degradation of muscle tissue. The other alternative is to consume a combination of plant-based proteins, such as pea and rice protein, to compliment the amino acid profile and create a more complete protein supplement. This would also reduce the need for ingesting excessively large boluses of protein per serving. Since aging has been associated with reduced appetite and food intake (35, 107), including insufficient protein ingestion (62, 86), smaller servings would likely be optimal for this population.

Furthermore, it has been suggested that plant-based proteins are more likely to be converted to urea. Tujioka and group provided amino acid mixtures representative of wheat, casein, and whole egg to rats for 10 days. In addition to reduced body weight gain, this group found increased liver concentrations of AA and resulting urea production from wheat compared to the animal-based AA mixtures (170). Since AA mixtures were provided, differing digestibility was not a factor, suggesting that the EAA content of the proteins are responsible for the urea production, rather than lower digestibility. In elderly humans, Yang et al. found increased leucine oxidation rates for 20 g soy protein compared to 20 g whey protein ($p = 0.002$) relative to lean body mass (182). This suggests that plant proteins are directed to fates other than protein synthesis compared to similar doses of animal-based protein. Taken together, it would seem that the EEA content of the protein is driving these differences.

Table 1 Leucine Amino Acid Reference Ratio (AARR) for Selected Protein Sources

Protein Source	**Leucine AARR**
Whey Protein Isolate	2.57
Whey Protein Concentrate	1.93
Milk Protein Concentrate	1.77
Pea Protein Concentrate	1.37
Soy Protein Isolate	1.29
Rice Protein Concentrate	1.11

Adopted from Rutherford 2015 (148)

Plant-based proteins contain lower lysine and/or methionine content than do animal-based proteins (49, 172), thus providing a suboptimal EEA content. For example, rice contains a low lysine (3.8%) content compared to animal-based proteins and human muscle, but a sufficient quantity of methionine (2.2%). Additionally, pea contains a low methionine content (1.6%), but a relatively high amount of lysine (6.3%). This provides additional support that combining plant-proteins with complimentary amino acid profiles, such as rice and pea, would provide a more optimal EAA content, and theoretically have a fate of protein synthesis rather than urea production. However, no work has been done examining protein synthesis, urea production, or functional outcomes with a combination of plant-based proteins.

2.2.2 Protein Timing

While protein dose per serving is an important factor in muscle reconditioning, other factors must be considered, such as protein timing. Anecdotally, strength athletes, bodybuilders, and weightlifters have consumed protein immediately following resistance training. This, perhaps, arose from the idea of an "anabolic window" following the bout of resistance exercise. This narrow window of opportunity has been described as the time period 45-60 minutes post-resistance training where protein must be consumed to gain the greatest benefit (149). Protein timing has been extensively studied. Previous work has examined daily protein distribution patterns (8, 95). In a well-controlled study, young, resistance trained individuals were provided with 80 g total whey protein isolate. Protein was distributed as a bolus of 40 g, 20 g, or 10 g distributed every 6, 3, and 1.5-hrs, respectively, over a 12-hour post-exercise recovery period. The 40g bolus induced the greatest insulin concentrations, aminoacidemia, and anabolic signaling response. Interestingly, the increases in FSR during the early recovery phase (1-4 hrs) were not different between the doses ($p > 0.05$). During the entire 12-hour recovery period, however, the 20 g distribution increased myofibril FSR by 31% and 48% ($p < 0.02$) compared to 10 g or 40 g, respectively (8). This suggests that while a threshold dose must be met per serving, nutrient timing plays a role in the synthetic response. This study also suggests that while acutely, total protein intake may be the primary contributor to muscle reconditioning, protein timing may play a more important role in chronic adaption and muscle reconditioning. In agreeance with these findings, Mamerow et al. examined the effects on men and women (36.9 ± 3.1 years old) of even distributions of moderate doses of protein throughout the day, or skewing protein ingestion

such that protein consumption is minimal in the morning and greatest in the evening, as is often seen in many countries. Using a crossover design with a 30-day washout period and 7 days of habituation to either ~30 g protein 3 times per day or an isoenergetic and isonitrogenous diet with 10 g protein at breakfast, 15 g protein at lunch, and 65 g protein at dinner. Using a primed constant infusion of labeled phenylalanine and muscle biopsies, the authors reported 24-hour mixed muscle FSR to be ~25% greater in the even distribution both at day 1 ($p = 0.003$) and day 7 ($p = 0.001$) of habituation compared to the skewed distribution (95). This study suggests that consuming moderate amounts of protein throughout the day is more beneficial for stimulating muscle protein synthesis than is the typical pattern of eating found in most countries. Taken in sum, those wishing to maximally stimulate the synthetic response should ingest a sufficient dose of protein in frequent intervals. The most popular timing of protein ingestion, and most frequently studied, is the time immediately pre- or post-workout. Previous studies have supported the consumption of protein immediately post-exercise (65, 141). When consuming ~18 g of whey protein or ~19 g of protein blend 1 hour following resistance exercise, anabolic signaling and FSR in the post exercise recovery period increased in healthy, young individuals (141). It has been suggested that acute MPS responses be viewed as a proxy for the muscle reconditioning response and recovery, rather than an estimation of hypertrophy (172). Similarly, post-exercise consumption of 30 g whey protein decreased fat mass and increased relative fat free mass to a greater extent than did carbohydrate or carbohydrate and protein combination. However, not all research has found similar findings (25, 60, 171). Hoffman et al. provided 42 g of a mixed protein beverage to strength trained men either immediately pre- and post-exercise or in the morning and evening, with no differences between groups for body composition, strength, or power after 10 weeks of resistance training (60). Candow and colleagues provided either a placebo or 0.3 g/kg of a protein supplement either before or after resistance training for 12 weeks to older, untrained men. While resistance training improved lean mass and strength, this study found no effect between groups (25). Verdijk et al. provided 10 g of casein hydrolysate before and after resistance training to healthy, elderly (72±2 years) for 12 weeks. This study also reported improvements from resistance training for muscle cross sectional area, muscle strength, and body composition, however, there were no differences between groups (171). It should be noted, however, that the doses provided were suboptimal and likely insufficient to maximally stimulate MPS, and therefore promote muscle reconditioning, especially in older individuals.

While the pre- and post-exercise timing window has been the most popular ingestion period, pre-sleep protein ingestion is a novel feeding opportunity which has been researched recently.

2.2.2.1 Night-Time Protein Feeding

Recent research has investigated the night-time protein feeding window just prior to sleep. In young and aging men, casein protein (40 g) was shown to be effectively digested and absorbed and to increase muscle protein synthesis during overnight recovery (53, 142). Similarly, a casein and carbohydrate mixed beverage consumed prior to sleep increased muscular strength ($p < 0.001$), cross sectional area ($p < 0.05$), and type II muscle fiber size ($p < 0.05$) following 12 weeks of a resistance training protocol compared to a noncaloric placebo (155). These studies support the notion that ingestion of a night-time protein meal can improve lean mass accrual. Work from our own lab has shown night-time protein feeding to have beneficial or non-detrimental effects on metabolism and improve morning satiety without altering lipolysis (78, 93, 120). Consumption of 30 g whey and casein protein prior to sleep increased ($p < 0.05$) resting energy expenditure (REE) in active men with no alteration in next morning satiety (93). REE was not changed when 30 g of casein protein was consumed prior to sleep in obese women, but did improve next morning satiety to a greater extent ($p < 0.05$) than did whey or carbohydrate (120). In young obese men, consumption of 30 g casein protein pre-sleep did not impede overnight or morning fat metabolism (78). It should be noted that recent work suggests that 30 g of pre-sleep casein protein can improve overnight whole-body protein balance compared to placebo ($p <0.001$) and those amino acids are incorporated into myofibrillar protein during the 7.5-hour overnight period. However, 30 g casein protein or 30 g casein protein + 2 g free leucine was insufficient to stimulate myofibrillar protein synthesis rates above placebo during the overnight period in recreationally active, young men (23 ± 1 years) when consumed 30 minutes pre-sleep (169). This work suggests that 30 g of pre-sleep protein is insufficient to stimulate marked increases in overnight MPS. As stated above, 40 g of pre-sleep protein has shown benefit acutely and chronically on muscle adaptation. While an optimal dose of pre-sleep protein has yet to be determined, it seems that at least 40 g of protein should be consumed pre-sleep. Taken in sum, night-time protein feeding may be an additional opportunity to provide nutrients for those wishing to increase body composition via increased MPS thus lean mass accrual, increased REE, and, improved next day satiety without alteration of fat metabolism.

While there is an abundance of research on the effects of pre-sleep protein on synthetic rate and metabolism, there is a dearth of knowledge regarding pre-sleep protein and muscle recovery. However, three recent studies have examined the effects of night-time protein feeding on muscle recovery following damaging resistance exercise. West et al. provided a protein free cookie with a 50:50 carbohydrate:fat composition (~70 kcal) plus either 25 g whey protein or calorie matched carbohydrate to healthy, trained young men immediately following a night-time whole body resistance training session prior to sleep (20:00). Net protein balance was enhanced over the 24-hour recovery period in the protein group, but not the carbohydrate group ($p = 0.036$). Interestingly, the carbohydrate group had greater protein synthesis rates than the protein group, but the protein group had reduced protein breakdown (174). This work also showed protein to have small to moderate effects on MVC (ES: 0.28), countermovement jump (ES: 0.49), and mean anaerobic power (ES: 0.49) at 10h and moderate effects of protein for MVC (ES 0.76), knee extension repetitions to failure (75% 1RM; ES: 0.44), and peak power (ES: 0.55) at 24-hrs compared to carbohydrate. Abbott et al. 2019 examined recovery of professional soccer players (male, age: 19 ± 1 yrs) following a night-time soccer match. Using a crossover design, this group provided either 40 g casein protein (CP) or 40 g carbohydrate (CHO) after the soccer matches and 30 minutes prior to sleep. There were no clear differences in work completed during the soccer matches between groups. CP had clear small to moderate benefits on attenuating reductions in countermovement jump height up to 36-hrs post-match, clear large benefits on attenuating reactive strength index up to 36-hrs post-match, and large benefit at 12-hrs post-match for reducing muscle soreness compared to CHO (2). Of note, these benefits were observed even with sufficient daily protein intakes in CP (1.86 ± 0.22 g/kg) and CHO (1.93 ± 0.27 g/kg). However, not all data supports a benefit of pre-sleep protein on recovery. Apweiler et al. sought to determine the effect of pre-sleep protein compared to a carbohydrate control on muscle recovery from exercise induced muscle damage. Young men and women performed 100 drop jumps in the morning and consumed either ~40 g casein protein (CP) or ~40 g carbohydrate (CHO) before night-time sleep. All participants consumed a post-workout protein and carbohydrate recovery drink immediately following exercise. While the exercise protocol was shown to induce low (~10-15%) reductions in force production, indicating damage, no differences were measured between CP and CHO and for men or women in countermovement jump, maximal isometric voluntary contraction, soreness or pain pressure threshold (7). This

study reported total daily protein intake to be sufficient for both CP (2.12 ± 0.51 g/kg) and CHO (1.60 ± 0.59 g/kg). It could be that differences between groups were not observed because total daily protein intakes were met. This agrees with work by Joy et al. (73) and Antonio et al. (6), but disagrees with Abbott et al. (2). One possibility for the lack of improvement in recovery with pre-sleep protein is the timing of the exercise (morning exercise for the Apweiler et al. study), which the authors cited as the likely reason for differing results from the work of Abbott et al.(2) and West et al.(174) (these groups utilized evening exercise). It should be noted that all of these studies used a calorie matched carbohydrate control, thus the argument could be made that pre-sleep carbohydrate is as effective as pre-sleep protein in healthy young men women at promoting improvements in body composition, performance, and muscle recovery rather than the conclusion that protein did not improve recovery. There is a dearth of information that compares eating to not eating at night before sleep, and how this may influence muscle recovery. Therefore, work is needed that utilizes a non-caloric placebo to determine if protein consumed before night-time sleep is in fact more effective than consuming nothing pre-sleep for improving muscle recovery following exercise induced muscle damage. It seems that dairy-based pre-sleep protein is beneficial for improving muscle recovery following night-time exercise bouts, but the efficacy is questioned if the training bout is performed earlier in the day. More work is needed to elucidate if pre-sleep protein is effective for improving muscle recovery following morning workouts, especially studies which control total daily protein intake. To date, all of the studies regarding pre-sleep protein on muscle recovery have used casein as the protein source. Future work should be done to investigate if other sources of protein consumed prior to night-time sleep are also effective at improving muscle recovery. Studies which utilize whey proteins during the pre-sleep feeding window should be conducted as whey protein has been shown to increase MPS, a marker of muscle reconditioning, to a greater extent than casein (22, 128, 161).

2.2.3 Protein Source

Various protein sources elicit different rates of hyperaminoacidemia in plasma (175). Sources such as whey and soy are termed “fast” as the post-consumption concentration of plasma amino acids is rapid. In contrast, sources such as casein are termed “slow” as the post-consumption aminoacidemia is slower and sustained longer. While plant-based protein products are gaining

popularity, this may not be optimal to animal- or dairy-based proteins. Little work has been done directly comparing the efficacy of varying sources of proteins.

The work that has been done in this area has examined fluid milk compared to soy protein. In resistance trained men, 500-mL (~18 g protein) of fluid milk and isonitrogenous, isocaloric, and macronutrient matched soy protein elevated amino acid uptake and FSR post-exercise ($p < 0.05$). However, intact milk protein elicited a 34% greater FSR ($p < 0.05$) during the 3-hour recovery period compared to soy (175). Using a similar supplementation protocol, work by the same group showed a greater hypertrophic response to chronic post-exercise consumption of intact milk protein following 12 weeks of resistance training compared to soy protein. In young, novice weightlifters 500-mL of fat-free fluid milk increased ($p < 0.05$) type II fiber cross sectional area and lean mass to a greater extent than did isonitrogenous, isocaloric, and macronutrient matched soy protein or carbohydrate control (55). Yang and colleagues compared differing doses of soy and whey protein isolate in older men. Neither 20 g nor 40 g of soy protein stimulated MPS at rest while both 20 g and 40 g of whey protein did. Further, 40 g but not 20 g of soy protein stimulated MPS post-exercise while both 20 g and 40 g of whey protein did ($p < 0.05$). It should be noted that the synthetic response to soy was less ($p < 0.001$) than that of the dairy-based whey protein (182). In a nutritionally controlled study design, which utilized unilateral leg exercise such that the contralateral leg served as a non-exercise control, Tang et al. provided ~20 g of either whey hydrolysate, casein, or soy protein to young, resistance trained men. Whey hydrolysate induced greater ($p < 0.05$) aminoacidemia at both 30- and 60-min post-ingestion compared to soy and casein. Resting mixed protein synthesis was greater ($p < 0.01$) in whey hydrolysate and soy compared to casein. Following exercise, whey hydrolysate increased ($p < 0.05$) MPS to a greater extent than did either soy or casein (161). Interestingly, it seems that whey hydrolysates may have beneficial effects on oxidative stress (137), inflammation (88), and exercise induced stress response (156).

While ingesting larger per serving doses of plant-based protein could be an option to mediate differences between anabolic responses of dairy- and plant- based protein, consuming portions of >40 g of a single plant protein per serving is hardly feasible, especially considering aging individuals have a potential for reduced appetite. It is possible that one could consume a

combination of plant-based proteins to obtain a more complete amino acid profile, thus, requiring a lower per dose serving to obtain similar benefits as dairy-based proteins. Unfortunately, no work has been done examining the effectiveness of plant-based protein combinations in response to resistance training. Further research is needed to determine the effectiveness of other plant- based sources of protein, such as rice and pea or a combination thereof, to dairy-based proteins such as whey and casein. To date, no studies have directly compared the effects of dairy- or plant- based protein combinations on muscle recovery or metabolism following a damaging bout of resistance exercise.

2.2.3.1 Protein Quality Evaluation

Protein digestibility amino acid score (PDCAAS) has traditionally been used to estimate protein quality. Recently, expert recommendation by the Food and Agriculture Organization of the United Nations (FAO) has been to replace PDCAAS with the digestible indispensable amino acid score (DIAAS). For a full overview of the expert consultation, readers are referred to the FAO report (40)FAO. Report of an FAO Expert Consultation, 2013). To summarize the key points, PDCAAS use fecal protein digestibility which, due to limitations of the method, may overestimate values of true protein digestibility especially with proteins of lower quality. To that note, ileal protein digestibility at the terminal ileum better represents true protein digestibility and thus should be used in estimating protein quality. Ileal protein digestibility should be determined in rank order of humans, pigs, and rats. Additionally, dietary amino acids should be treated as individual nutrients and data should be reported in food tables, when possible. Though the expert opinion is to replace PDCAAS with DIAAS, and fecal protein digestibility with terminal ileal digestibility, further research is needed for various proteins and protein sources. Table 2 lists the PDCAAS and DIAAS of protein sources relevant to this thesis (148).

2.3 Conclusion

Resistance exercise is recommended for individuals of all ages to increase muscular strength and power, improve body composition, and improve performance of activities of daily living. However, novel exercise or exercise of unaccustomed intensity can lead to DOMS, inflammation, decreased performance, and metabolic alterations. Protein supplementation has been suggested to mediate the negative effects of exercise induced muscle damage and enhance

muscle recovery. Most work has examined the effectiveness of protein consumption either pre- or post-exercise. However, little work has been completed examining the effects of protein on muscle recovery in middle-aged individuals. Further, little is known about the effectiveness of dairy-based protein compared to plant-based protein sources, either in isolation or in combination, on younger or older populations. No studies have directly compared the effects of a dairy-based protein and plant-based protein in combination, such as a rice and pea protein combination on muscle recovery in younger or older populations. A novel area of research has been pre-sleep protein ingestion. This timing of protein ingestion represents an additional feeding window, which has shown benefit on MPS with possible benefit, but no harmful effects, on lipolysis. Additionally, recent work suggests casein protein consumed before bed can improve muscle recovery in younger individuals. Despite evidence supporting benefits of pre-sleep protein, no studies identify if the type of protein consumed pre-sleep influences muscle recovery. Therefore, the purpose of this study was to compare the effectiveness of dairy- or a combination of plant-based pre-sleep protein on muscle damage, metabolism, and performance following acute eccentric resistance exercise in middle-aged men.

Table 2 DIAAS and PDCAAS of Select Protein Sources

Protein Source	**DIAAS**	**PDCAAS**
Milk Protein Concentrate	1.18	1.00
Whey Protein Isolate	1.09	1.00
Whey Protein Concentrate	0.973	1.00
Soy Protein Isolate	0.906	1.00
Pea Protein Concentrate	0.822	0.893
Rice Protein Concentrate	0.371	0.419

Adopted from Rutherford 2015 (148)

CHAPTER 3

METHODOLOGY

3.1 Participants

Middle-aged, recreationally active healthy men (40-64 years) were recruited to participate in this study (N=32). Recreationally active was defined as engaging in physical activity on ≥ 2 days of the week for the past 6 months. Participants were excluded if they engaged in eccentric specific exercise of the lower limbs in the past 6 months, chronically took any anti-inflammatory medication, or had a musculoskeletal injury in the past 3 years that would inhibit performance and completion of this study. Additionally, participants were excluded if they had uncontrolled cardiovascular disease (CVD) or metabolic disorders, if they had a BMI ≥ 30 kg/m^2 and a body fat percentage ≥ 28%, or if they had allergies to dairy products. Lastly, participants were excluded if they smoke or quit smoking in the past 6 months. A washout period was required if participants were taking any nutritional supplements. All participants were informed of the procedures and risks associated with participating and signed an informed consent indicating their understanding. All procedures were approved by the Florida State University Institutional Review Board prior to testing (IRB00000446).

3.2 Familiarization

Participants arrived at the Institute of Sports Sciences and Medicine (ISSM) at Florida State University in the overnight fasted state between the hours of 0500 and 0900. On **visit 1 (day -7)**, baseline measurements of height and weight were taken using a digital scale (Detecto Scale Company, Webb City, MO) and a wall mounted stadiometer (Seca Corporation, Chino, CA), respectively. Resting metabolic rate (RMR), body composition using dual energy x-ray absorptiometry (DXA; Hologic Model DPX-IQ, GE Medical Systems), isokinetic and isometric maximal voluntary contraction (MVC) using an isokinetic dynamometer (Biodex Medical Systems, Shirley, New York) was performed. Participants were instructed how to record their dietary intake and the MyFitnessPal app was downloaded for the participant. On **visit 2 (day -4)**, RMR and MVC testing was performed. A warm-up consisting of 5 minutes of treadmill walking at a self-selected pace and a 1.0% grade was completed prior to all exercise on all visits.

3.3 Experimental Design

A stratified, randomized, double blind, placebo-controlled study design was utilized. Participants were stratified by both age and body fat percentage then assigned (N= 8/group) to either whey hydrolysate (WH), whey protein isolate (WP), rice and pea combination (RP), or a flavor matched, non-caloric placebo (PL) immediately post-eccentric exercise (25 g) and 30-minutes pre-sleep (40 g) on the eccentric exercise day (**visit 3, day 0**) and the two subsequent experimental trial days (**+24, +48**). Protein supplements were weighed, packaged, and labeled by a researcher not otherwise involved in this study. Prior to leaving the laboratory, participants received a supplement package and an opaque bottle containing ~350 ml of water with instruction as to how and when to consume the beverage. Participants were instructed to write the time of consumption on the provided package and bring the empty package to the laboratory in the morning to indicate compliance.

3.4 Exercise Protocol

On **visit 3 (day 0)**, participants arrived at ISSM in the overnight fasted state between the hours of 0500 and 0900. Upon arrival, RMR testing was performed. Following RMR, baseline measurements of perceived muscle soreness were assessed using a visual analogue scale as described below. Then, baseline thigh circumference measurements of the dominant limb were performed. A baseline blood sample was collected as described below. Participants then performed 5 sets of 15 repetitions of bilateral maximal voluntary eccentric contractions of the knee extensors and knee flexors, respectively, for 5 sets of 15 repetitions at 60°/s on an isokinetic dynamometer. Two minutes of rest was given between sets and 5 minutes between each leg. This specific protocol has been shown to induce muscle damage (163) and similar protocols have been shown safe in older men (85, 87). Visual feedback was provided during the entirety of the exercise bout and verbal encouragement was provided by the researchers to promote maximal effort. The post-exercise supplement was consumed within 10 minutes of completion of the exercise protocol under supervision in the laboratory following post-exercise blood collection (+0). Ten minutes following ECC, isokinetic MVC was measured at angular velocities of 60°/s, 180°/s, and 300°/s and isometric MVC was measured at 60°. RMR testing was performed at 45-min post-exercise. A whole food meal (Vale Food Co., Tallahassee, Fl.; ~400 calories, 76 g

CHO, 8 g FAT, 5 g PRO) was provided and consumed under supervision in the laboratory 2-hrs following ECC. Blood samples were collected immediately post and at 4 and 6-hrs post-ECC.

3.5 Post-Exercise Testing

On **visits 4, 5**, and **6**, participants returned to the laboratory at 24, 48, 72-hrs post-ECC in the overnight fasted state between the hours of 0500 to 0900. RMR, muscle soreness, and thigh circumference testing was performed. A fasting blood sample was collected, then isokinetic MVC was measured at angular velocities of 60°/s, 180°/s, and 300°/s and isometric MVC was measured at 60° on the dominant limb.

3.6 Resting Metabolic Rate

Participants arrived at the laboratory in the morning in the overnight fasted state. Participants were asked to refrain from caffeine consumption for 12-hrs and physical activity and alcohol consumption for 24-hrs prior to all metabolic measurements. Upon arrival, height and weight measurements were recorded. Testing was performed in a dark, climate-controlled room (20 - 23ºC). RMR was measured with computerized open-circuit indirect calorimetry ParvoMedics TrueOne 2400 metabolic cart, Sandy, UT, USA) using a fitted hood. Gas exchange was measured continuously for 30 minutes and measurements recorded in the last 20 minutes were used for data analysis. Prior to testing, calibrations were performed with a flow meter using a 3-liter syringe and with gas analyzers using verified gases of known concentrations.

3.7 Muscle Soreness

Participants were seated on a bench and asked to perform a full knee extension and knee flexion. They were asked to rate their perceived soreness in the lower extremities using an anchored 100 mm visual analogue scale at pre, 24, 48, and 72-hrs post-ECC prior to blood sampling. The scale was labeled with “no soreness” at 0 mm and “extreme soreness” at 100 mm. Participants were instructed to make a vertical line along the scale to indicate perceived soreness in the muscles of the lower limb during the entire movement.

3.8 Thigh Circumference

A Gullick tape measure was used to quantify thigh circumference on the dominant leg of each participant. Circumference was measured while placing the foot on a bench with the knee bent at a 90° angle. A mark was made at the midpoint between the proximal border of the patella and the intersection of the inguinal crease and anterior midline of the thigh to ensure measurements were taken in the same location during each trial.

3.9 Blood Analysis

Blood was collected from an antecubital vein (10 ml) at pre, 0, 4, 6, 24, 48, and 72-hrs post-exercise into vacutainer tubes lined with EDTA (Becton, Dickinson & Company, New Jersey). Blood was centrifuged (Thermo Scientific, Waltham, MA) for 15 minutes at 3500 rpm at 4º C. Plasma aliquots (300-500 µL) were transferred into microtubes and stored at -80º C for later analysis. CRP, IL-10, insulin (Cat. Nos. SCRP00, DINS00, SS100C; R&D Systems, Minneapolis, MN), and CK (Cat. No. MAK116, Sigma Aldrich, St. Louis, MO) concentrations were determined by immunoassay according to manufacturer instructions. Glucose and IL-6 concentrations were measured using a Beckman Coulter DxC600i. Sensitivities were 0.022 ng/mL, 0.17 pg/mL, 2.15 pmol/L, 30 U/L, 5 mg/dL, and 0.5 pg/mL, respectively. Glucose and insulin concentrations were used to calculate HOMA-IR.

3.10 Maximal Voluntary Contraction

Testing was performed on the Biodex isokinetic dynamometer (Biodex Medical Systems, Shirley, NY). The participant was positioned so that the backrest was firmly against the back and the hips flexed at 90°. Hip, thigh, and chest straps were tightened so the participant was firmly secured into place. The lever arm was aligned visually at the axis of rotation of the knee joint at the lateral epicondyle. The lever arm was extended so that the inferior rim of the ankle pad was contacting the tibia just above the malleoli of the ankle. The positioning was recorded for each participant and utilized for all subsequent testing. The range of motion for extension and flexion was set. The leg was extended, locked into place, and the weight of the limb was recorded.

Maximal concentric-concentric torque was measured in the dominant knee extensors and flexors at angular velocities of 60°/s (ISOK60), 180°/s (ISOK180), and 300°/s (ISOK300). Five repetitions were performed for extension (ext) and flexion (flex), respectively, for all velocities.

Sixty seconds of rest was given between changing velocities. The greatest peak torque at each velocity for each movement was recorded and used in data analysis for isokinetic MVC.

Maximal isometric contraction (ISOM) was measured while still secured in the same position. The participant's limb was moved to 60°. Three total repetitions were performed for isometric MVC for each extension and flexion. Repetitions were performed as 5 seconds MVC extension followed by 5 seconds of rest then 5 seconds of MVC flexion until all 3 repetitions were completed. The greatest MVC for each movement was recorded as peak torque and used in data analysis. Baseline measurements during visits 1 and 2 utilized both limbs to determine maximal concentric torque of both limbs in order to assess effort during the eccentric exercise on Day 0. Only the dominant leg was tested during post-exercise time points.

3.11 Nutritional Control

Caloric needs were determined from RMR data adjusted with an activity factor of 1.375. Participants received a standardized diet of 15% PRO, 55% CHO, and 30% fat (Vale Food Company, Tallahassee, FL) 2 days prior to and during all exercise and experimental days (+24h, +48h, +72h**)**.

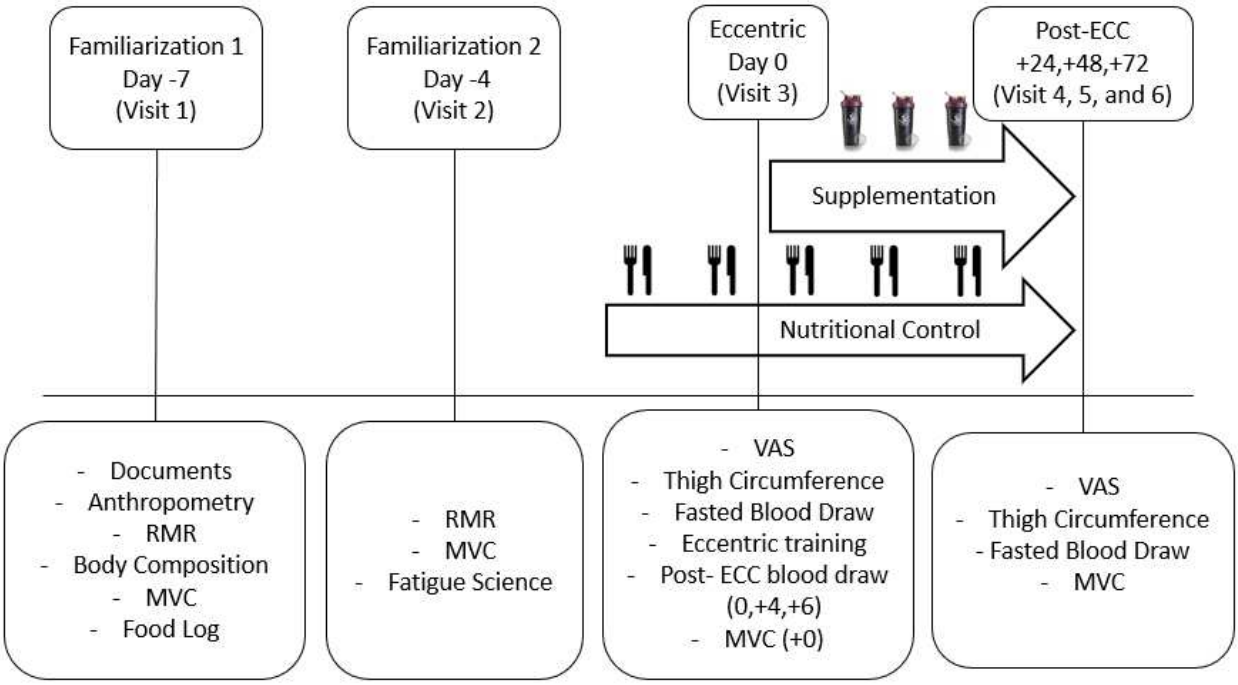

Figure 1. Overview of Study Procedures

3.12 Statistics

Participants were stratified into groups based on age and body composition to ensure homogeneity of the sample groups. A priori power analysis for CK, a primary outcome of our study, was performed based on a study investigating plant-based protein on muscle recovery following damaging exercise which reported a mean of 878.2 ± 72.12 vs. 1133.74 ± 152.23 for protein vs. placebo, respectively and an effect size of 0.73 for CK AUC (179). Using an α level of 0.05, β of 0.8 with 4 groups and 7 time points, it was determined that the required sample size to fully power this research was two participants per group (G Power version 3.1.9.2, Düsseldorf, Germany). Due to the small sample size determined for CK, a second a priori power analysis was performed with a much more conservative effect size to ensure that the study was adequately powered. To observe changes if they are present for CK, a sample size of 7 per group was required with an α level of 0.05, β of 0.8, and a small to moderate effect size of 0.25. Additionally, a priori power analysis for isometric peak torque was performed based on a similar study investigating pre-sleep protein on muscle recovery. Though raw data were not included, the authors reported an effect size of 0.28 for isometric peak torque at 10-hrs (174). Using an α level of 0.05 and β of 0.8 with 4 groups and 5 time points, it was determined that per group requirement to observe changes was 7 participants per group. A one-way ANOVA was utilized to examine baseline participant characteristics between groups. Within subjects and between treatments repeated measures ANOVA (RMANOVA) was used to determine time x treatment effects for all outcome variables. All measures contained 4 groups. Blood variables had 7 time points, muscle soreness and thigh circumference had 4 time points, and muscle function had 5 time points. Significant main effects were further investigated using Bonferroni pairwise comparisons. Bonferroni *post hoc* analysis was performed to determine between group differences for significant time x treatment interactions. If sphericity was violated, Greenhouse-Geisser corrections were used. All group x time interactions are reported as F ($df_{treatment}$,df_{error}) = F statistic, p-value, partial eta squared (η_p^2). For ηp^2, effect size values were qualified as follows: small = 0 - 0.02, medium = 0.02 - 0.13, large = 0.13 - 0.26. Statistical analysis was completed using SPSS (IBM SPSS Statistics for Windows, version 25; IBM Corp.). Significance was set at $p < 0.05$. Data are reported as mean ± standard error of the mean (SEM).

CHAPTER 4

RESULTS

4.1 Participant Characteristics

Twenty-eight middle-aged recreationally active males were recruited from the Tallahassee, Florida community and volunteered to participate in the study. One participant dropped the study due to diagnosis of rhabdomyolysis prior to completion of the study. Those data were removed from analysis. A second participant was diagnosed with rhabdomyolysis after completion of the study. Those data were included in final analyses. While 32 participants were to be recruited, the study was terminated prior to the anticipated 32 total participants due to safety concerns from the exercise protocol. Thus, 27 individuals were included in final analyses. There were no significant differences at baseline between groups for age, height, weight, body fat %, or lean mass (see Table 3).

Table 3 Participant Characteristics

	Whey Hydrolysate	Whey Isolate	Rice/Pea	Placebo	Total	p-value
N	9	6	6	6	27	
Age, yr	57 ± 2	53 ± 3	56 ± 2	52 ± 4	55 ± 1	0.655
Height, cm	179 ± 3	180 ± 3	179 ± 2	180 ± 3	179 ± 1	0.965
Weight, kg	86.1 ± 4.3	83.9 ± 4.4	79.1 ± 4.9	78.7 ± 5.5	82.4 ± 2.3	0.613
Lean Mass, kg	61.7 ± 1.9	60.7 ± 2.5	57.2 ± 3.2	55.9 ± 3.2	59.2 ± 1.3	0.346
Body fat, %	24 ± 2	23 ± 2	23 ± 1	24 ± 2	23 ± 1	0.967

4.2 Nutrient Intake

Mean energy intake for all participants during the 5 days of standardized nutrition was 2351 ± 60 kcals (PRO: 1.08 ± 0.02 g/kg, CHO: 323 ± 8 g, FAT: 78 ± 2 g). There were no differences between treatments for calories, PRO, PRO/kg, CHO, or fat intake (see Table 4).

Table 4 Standardized Nutrition

	Whey Hydrolysate	Whey Isolate	Rice/Pea	Placebo	Total	p-value
Total Calories	2405 ±113	2439 ± 136	2293 ± 102	2240 ± 135	2351 ± 60	0.654
PRO, g	90 ± 4	91 ± 5	86 ± 4	84 ± 5	88 ± 2	0.654
PRO, g/kg	1.05 ± 0.04	1.10 ± 0.06	1.10 ± 0.06	1.08 ± 0.06	1.08 ± 0.02	0.894
CHO, g	331 ± 16	335 ± 19	315 ± 14	308 ± 19	323 ± 8	0.654
Fat, g	80 ± 4	81 ± 5	76 ± 3	75 ± 5	78 ± 2	0.654

4.3 Blood Biomarkers

4.3.1 CK

Intra and Inter assay CVs for CK were 3.25% and 2.92%, respectively. One-way ANOVA indicated that there were no differences between groups at baseline ($p = 0.147$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 7 time points (pre, post, +4, +6, +24, +48, +72-hrs) indicated no significant group x time interactions ($F_{3.016, 21.113} = 0.184$, $p = 0.907$, $\eta_p^2 = 0.026$, $n = 25$) or main effects of group ($F_{3, 21} = 0.275$, $p = 0.843$, $\eta_p^2 = 0.038$. There was a main effect of time ($F_{1.005, 21.113} = 62.385$, $p < 0.001$, $\eta_p^2 = 0.748$). Post hoc testing revealed that CK increased at +4-hrs ($p < 0.001$) and remained elevated at all time points compared to baseline, with a significant increase from all other time points at +72-hrs (see Figure 2).

4.3.2 IL-6

One-way ANOVA indicated that there were no differences between groups at baseline ($p = 0.535$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 7 time points (pre, post, +4, +6, +24, +48, +72-hrs) indicated no significant group x time interactions ($F_{9.961, 69.728} = 0.0.854$, $p = 0.579$, $\eta_p^2 = 0.109$, $n = 25$) or main effects of group ($F_{3, 21} = 0.422$, $p = 0.739$, $\eta_p^2 = 0.057$). There was a main effect of time ($F_{3.320, 69, 728} = 7.633$, $p < 0.001$, $\eta_p^2 = 0.267$). Post hoc testing revealed significantly greater IL-6 concentrations at +6-hrs compared to baseline ($p = 0.002$). IL-6

returned to baseline at +24-hrs and trended to increase again at +72-hrs compared to baseline ($p = 0.089$; see Figure 3).

4.3.3 IL-10

All measured concentrations were below the valid assay range (0.8 pg/mL). Thus, these data were not analyzed.

4.3.4 CRP

Intra and Inter assay CVs for CRP were 2.98% and 2.23%, respectively. One-way ANOVA indicated that there were no differences between groups at baseline ($p = 0.577$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 7 time points (pre, post, +4, +6, +24, +48, +72-hrs) indicated no significant group x time interactions ($F_{4.779, 33.453} = 1.315$, $p = 0.282$, $\eta_p^2 = 0.158$) or main effects of group ($F_{3, 21} = 1.080$, $p = 0.379$, $\eta_p^2 = 0.134$). There was a main effect of time ($F_{1.593, 33.453} = 5.493$, $p = 0.013$, $\eta_p^2 = 0.207$). Post hoc testing revealed that CRP concentrations were significantly elevated immediately after exercise. No other time points were significantly different from baseline (see Figure 3).

4.3.5 HOMA-IR

Intra and Inter assay CVs for insulin were 2.81% and 0.98%, respectively. One-way ANOVA indicated that there were no differences between groups at baseline ($p = 0.306$; $n = 27$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 4 time points (pre, +24, +48, +72-hrs) indicated no significant group x time interactions ($F_{7.037, 53.950} = 1.559$, $p = 0.167$, $\eta_p^2 = 0.169$), main effects of group ($F_{3, 23} = 1.183$, $p = 0.338$, $\eta_p^2 = 0.134$), or main effects of time ($F_{2.346, 53.950} = 1.005$, $p = 0.383$, $\eta_p^2 = 0.042$; see Figure 2).

4.4 Muscle Function

4.4.1 ISOMext

One-way ANOVA indicated that there were no differences between groups for baseline power ($p = 0.867$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 5 time points (pre, post, +24, +48, +72-hrs) indicated no significant group x time interactions ($F_{8.191, 62.801} = 1.291$, $p = 0.263$, $\eta_p^2 = 0.144$). There were no significant main group effects ($F_{3, 23} = 0.227$, $p = 0.876$, $\eta_p^2 = 0.029$).

There were significant main time effects following the exercise bout ($F_{2.730,62.801} = 10.366$, $p < 0.001$, $\eta_p^2 = 0.311$). Post hoc tests indicated that peak torque was reduced immediately post-ECC and remained reduced compared to baseline until returning to pre-ECC values at +72-hrs (see Figure 4 and Table 5).

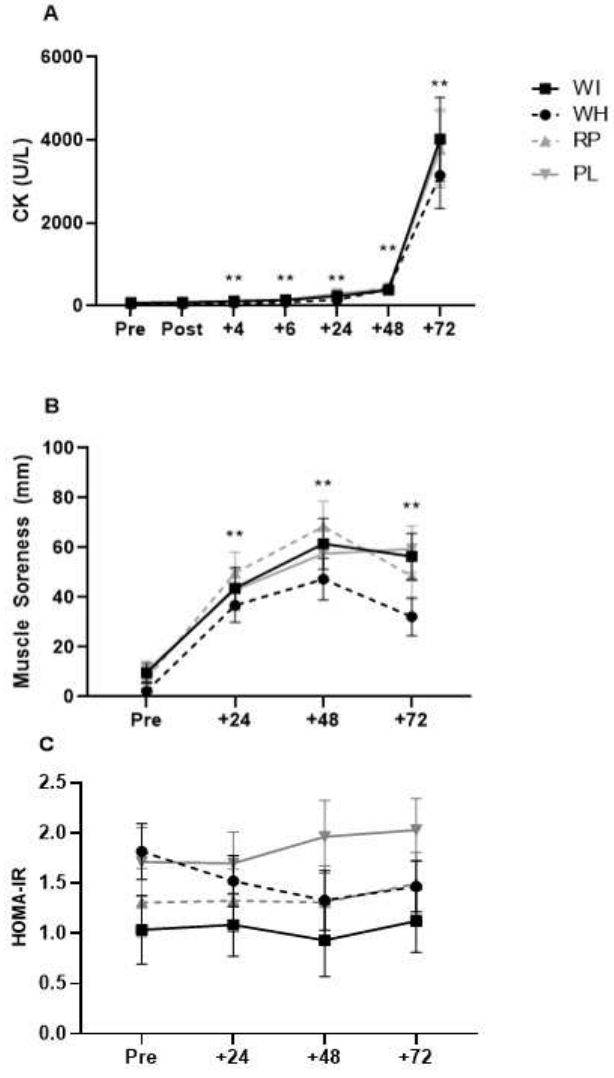

*Figure 2 Muscle Damage Markers A) Creatine Kinase B) Muscle Soreness C) HOMA-IR; ** indicates significant time effect from baseline ($p \leq 0.001$)*

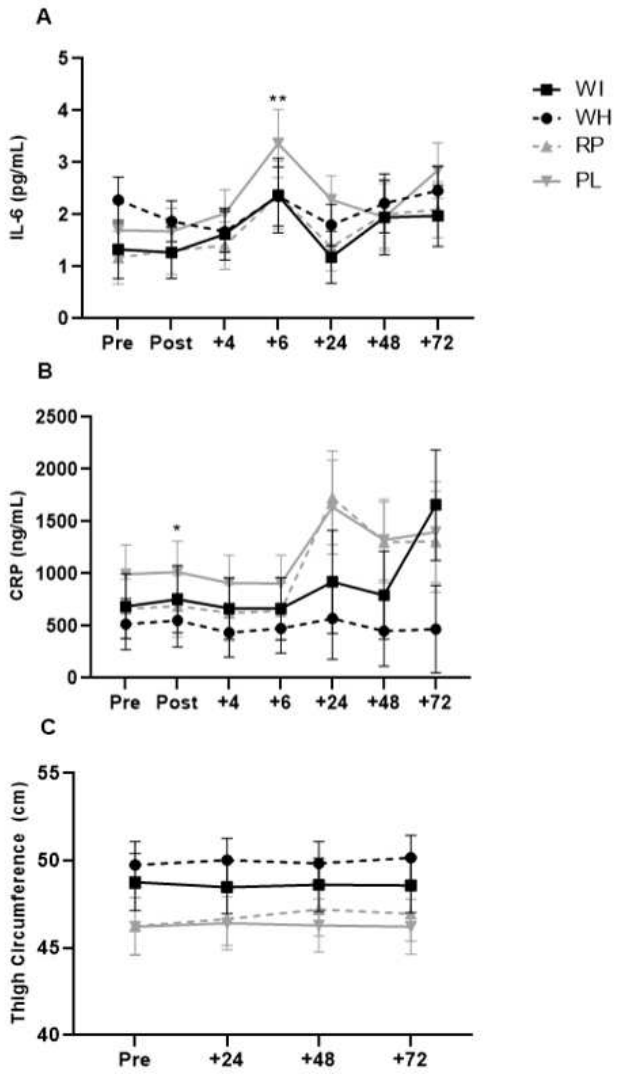

*Figure 3 Inflammatory Response A) IL-6 B) CRP C) Thigh Circumference; ** indicates significant time effect from baseline ($p \leq 0.001$); * indicates significant time effect from baseline ($p \leq 0.05$).*

4.4.2 ISOMflex

One-way ANOVA indicated that there were no differences between groups for baseline power ($p = 0.675$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 5 time points (pre, post, +24, +48, +72-hrs) indicated no significant group x time interactions ($F_{8.524, 65.347} = 1.031$, $p = 0.424$, $\eta_p^2 = 0.118$). There were no significant main group effects ($F_{3, 23} = 0.317$, $p = 0.819$, $\eta_p^2 = 0.040$). There were significant main time effects following the exercise bout ($F_{2.841,65.347} = 34.134$, $p < 0.001$, $\eta_p^2 = 0.597$). Post hoc testing indicated that peak torque was reduced at all time points following the exercise bout and did not return to pre-ECC values at +72-hrs (see Figure 4 and Table 5).

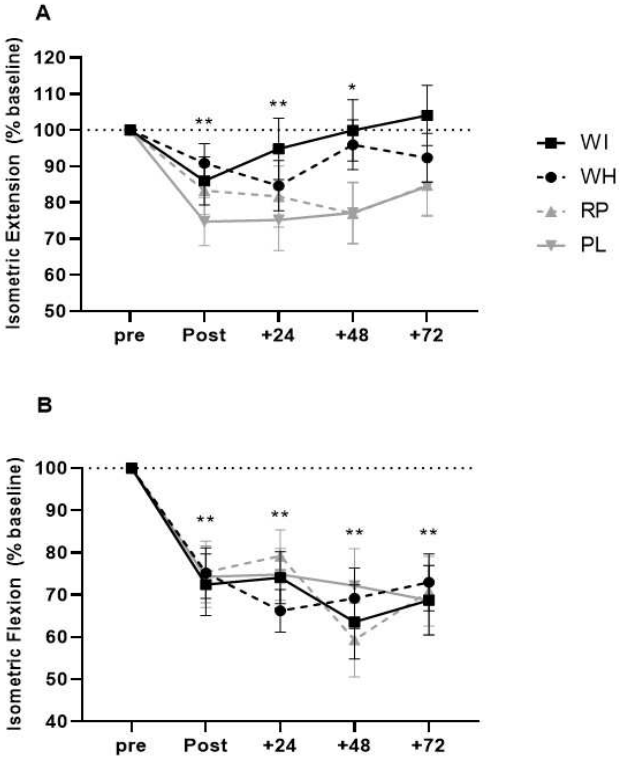

*Figure 4 Isometric Muscle Function A) Isometric extension; B) Isometric flexion; Raw data was analyzed for statistical purposes and represented as percent of baseline values; ** indicates significant time effect from baseline ($p \leq 0.01$); * indicates significant time effect from baseline ($p \leq 0.05$).*

4.4.3 ISOK60ext

One-way ANOVA indicated that there were no differences between groups for baseline power ($p = 0.632$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 5 time points (pre, post, +24, +48, +72-hrs) indicated no significant group x time interactions ($F_{7.510, 57.573} = 1.593$, $P = 0.151$, $\eta_p^2 = 0.172$). There were no significant main group effects ($F_{3, 23} = 0.784$, $p = 0.515$, $\eta_p^2 = 0.093$). There were significant main time effects following the exercise bout ($F_{2.503,57.573} = 19.258$, $p < 0.001$, $\eta_p^2 = 0.456$). Post hoc testing indicated that peak torque was reduced at all time points following the exercise bout and did not return to pre-ECC values at +72-hrs (see Figure 5 and Table 5).

4.4.4 ISOK60flex

One-way ANOVA indicated that there were no differences between groups for baseline power ($p = 0.842$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 5 time points (pre, post, +24, +48, +72-hrs) indicated no significant group x time interactions ($F_{6.417, 49.196} = 0.727$, $P = 0.639$, $\eta_p^2 = 0.087$). There were no significant main group effects ($F_{3, 23} = 0.827$, $p = 0.827$, $\eta_p^2 = 0.037$). There were significant main time effects following the exercise bout ($F_{2.139,49.196} = 39.055$, $p < 0.001$, $\eta_p^2 = 0.629$). Post hoc testing indicated that peak torque was reduced at all time points following the exercise bout and did not return to pre-ECC values at +72-hrs (see Figure 5 and Table 5).

4.4.5 ISOK180ext

One-way ANOVA indicated that there were no differences between groups for baseline power ($p = 0.864$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 5 time points (pre, post, +24, +48, +72-hrs) indicated no significant group x time interactions ($F_{8.373, 64.193} = 1.084$, $P = 0.386$, $\eta_p^2 = 0.124$). There were no significant main group effects ($F_{3, 23} = 0.630$, $p = 0.603$, $\eta_p^2 = 0.076$). There were significant main time effects following the exercise bout ($F_{2.791,64.193} = 8.835$, $p < 0.001$, $\eta_p^2 = 0.278$). Post hoc testing indicated that peak torque was reduced at all time points following the exercise bout and did not return to pre-ECC values at +72-hrs (see Figure 5 and Table 5).

4.4.6 ISOK180flex

One-way ANOVA indicated that there were no differences between groups for baseline power ($p = 0.945$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 5 time points (pre, post, +24, +48, +72-hrs) indicated no significant group x time interactions ($F_{7.688, 58.945} = 1.384$, $p = 0.225$, $\eta_p^2 = 0.153$). There were no significant main group effects ($F_{3, 23} = 0.584$, $p = 0.631$, $\eta_p^2 = 0.071$). There were significant main time effects following the exercise bout ($F_{2.563,58.945} = 10.576$, $p < 0.001$, $\eta_p^2 = 0.315$). Post hoc testing indicated that peak torque was reduced at all time points following the exercise bout and did not return to pre-ECC values at +72-hrs (see Figure 5 and Table 5).

4.4.7 ISOK300ext

One-way ANOVA indicated that there were no differences between groups for baseline power ($p = 0.741$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 5 time points (pre, post, +24, +48, +72-hrs) indicated no significant group x time interactions ($F_{7.730, 59.260} = 0.593$, $p = 0.774$, $\eta_p^2 = 0.072$). There were no significant main group effects ($F_{3, 23} = 0.720$, $p = 0.550$, $\eta_p^2 = 0.086$). There were significant main time effects following the exercise bout ($F_{2.577, 59.260} = 7.001$, $p < 0.001$, $\eta_p^2 = 0.233$). Post hoc tests indicated that peak torque was reduced immediately post-ECC and remained reduced compared to baseline until returning to pre-ECC values at +72-hrs (see Figure 5 and Table 5).

4.4.8 ISOK300flex

One-way ANOVA indicated that there were no differences between groups for baseline power ($p = 0.724$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 5 time points (pre, post, +24, +48, +72-hrs) indicated no significant group x time interactions ($F_{12, 92} = 0.617$, $p = 0.822$, $\eta_p^2 = 0.075$). There were no significant main group effects ($F_{3, 23} = 1.092$, $p = 0.372$, $\eta_p^2 = 0.125$). There were significant main time effects following the exercise bout ($F_{4, 92} = 6.102$, $p < 0.001$, $\eta_p^2 = 0.210$). Post hoc tests indicated that peak torque was reduced +24-hrs following the exercise bout and did not return to pre-ECC values at +72-hrs (see Figure 5 and Table 5).

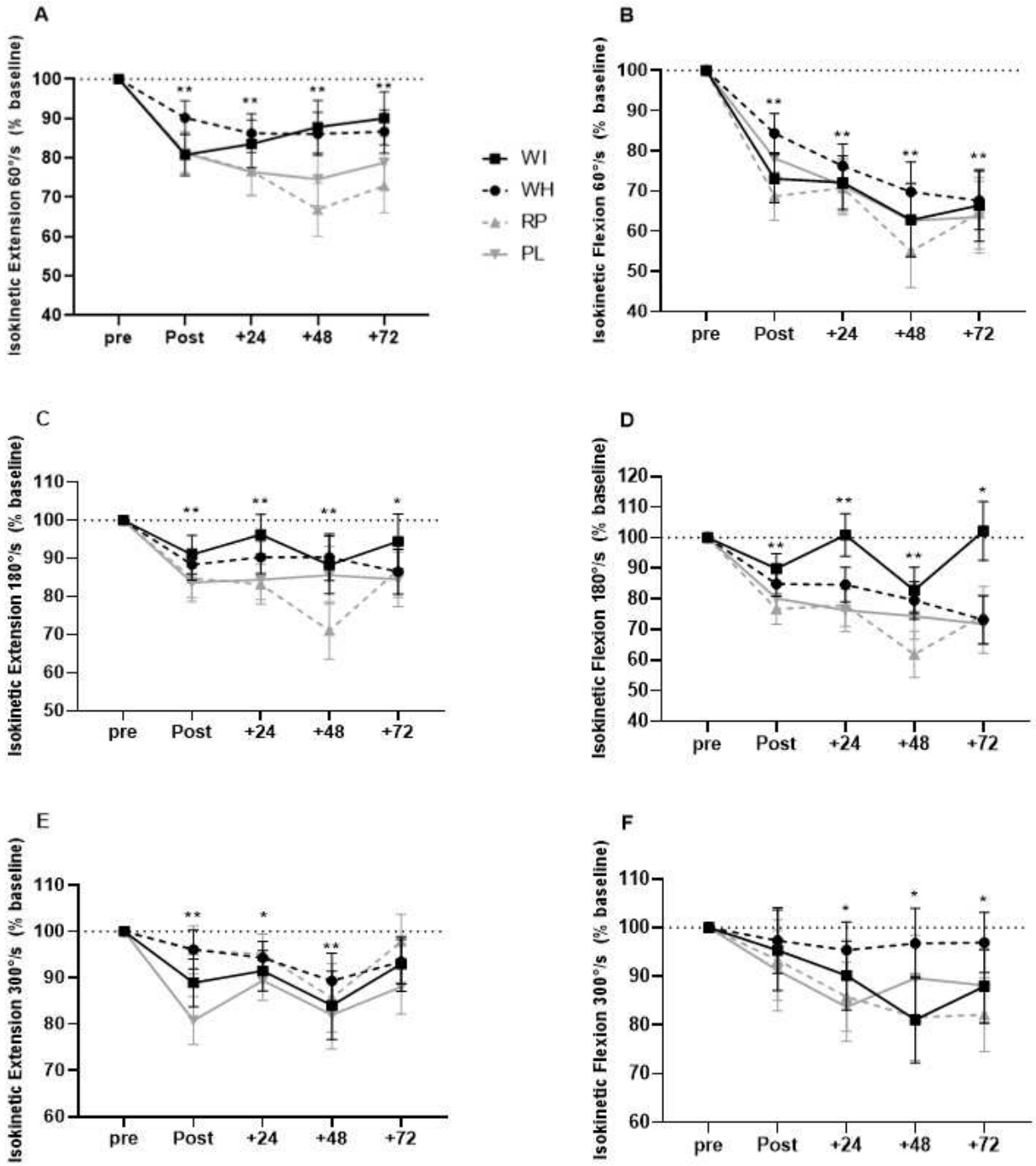

*Figure 5 Isokinetic Muscle Function A) Isokinetic extension 60°/s B) Isokinetic flexion 60°/s C) Isokinetic extension 180°/s D) Isokinetic flexion 180°/s E) Isokinetic extension 300°/s F) Isokinetic flexion 300°/s; Raw data was analyzed for statistical purposes and represented as percent of baseline values ** indicates significant time effect from baseline ($p \leq 0.01$); * indicates significant time effect from baseline ($p \leq 0.05$).*

Table 5 Raw Data for Muscle Function

Variable	Pre-exercise	Post (+0)	+24	+48	+72
ISOMext (Nm)					
WI	182.9 ± 15.4	152.9 ± 12.6	168.6 ± 15.4	175.0 ± 14.2	184.6 ± 18.0
WH	182.5 ± 10.3	164.3 ± 10.3	153.5 ± 12.6	173.7 ± 11.6	169.5 ± 14.6
RP	193.3 ± 15.4	157.7 ± 12.6	152.1 ± 15.4	144.3 ± 14.2	161.4 ± 18.0
PL	196.4 ± 15.4	144.8 ± 12.6	146.2 ± 15.4	148.5 ± 14.2	163.0 ± 18.0
ISOMflex (Nm)					
WI	86.2 ± 7.9	64.9 ± 8.9	66.7 ± 8.4	57.9 ± 9.1	60.6 ±8.7
WH	91.9 ± 6.5	68.7 ± 7.2	60.8 ± 6.9	64.0 ± 7.4	67.2 ± 7.1
RP	95.3 ± 7.9	69.1 ± 8.9	72.8 ± 8.4	51.4 ± 9.1	63.0 ± 8.7
PL	99.7 ± 7.9	73.8 ± 8.9	74.0 ± 8.4	70.9 ± 9.1	68.4 ± 8.7
ISOK60ext (Nm)					
WI	173.9 ± 10.9	140.8 ± 12.8	146.2 ± 14.0	152.1 ± 11.4	156.5 ± 13.8
WH	156.5 ± 8.9	142.1 ± 10.5	134.9 ± 11.4	134.2 ± 9.3	137.6 ± 11.3
RP	169.3 ± 10.9	136.8 ± 12.8	130.1 ± 14.0	108.6 ± 11.4	120.4 ± 13.9
PL	163.1 ± 10.9	132.0 ± 12.8	124.3 ± 14.0	120.6 ± 11.4	127.0 ± 13.9
ISOK60flex (Nm)					
WI	96.1 ± 8.0	71.1 ± 7.3	70.6 ± 8.4	61.5 ± 7.5	63.9 ± 8.5
WH	87.6 ± 6.5	72.8 ± 6.0	66.9 ± 6.9	60.1 ± 6.2	59.2 ± 7.0
RP	93.7 ± 8.0	62.6 ± 7.3	63.9 ± 8.4	43.5 ± 7.5	56.0 ± 8.5
PL	89.5 ± 8.0	70.0 ± 7.3	63.9 ± 8.4	56.0 ± 7.5	56.8 ± 8.5
ISOK180ext (Nm)					
WI	115.9 ± 8.4	104.5 ± 9.0	111.3 ± 9.2	102.8 ± 9.5	110.1 ± 11.3
WH	123.2 ± 6.9	109.5 ± 7.3	111.2 ± 7.6	110.5 ± 7.7	106.5 ± 9.2
RP	115.1 ± 8.4	97.9 ± 9.0	97.0 ± 9.2	79.2 ± 9.5	101.4 ± 11.3
PL	119.4 ± 8.5	99.2 ± 9.0	100.1 ± 9.2	101.3 ± 9.5	100.2 ± 11.3
ISOK180flex (Nm)					
WI	63.5 ± 6.5	56.4 ± 5.3	62.8 ± 6.4	52.0 ± 6.4	66.6 ± 10.0
WH	68.3 ± 5.3	57.3 ± 4.3	57.9 ± 5.3	54.5 ± 5.2	50.8 ± 7.9
RP	65.1 ± 6.5	49.4 ± 5.3	50.4 ± 6.4	37.7 ± 6.4	48.3 ± 9.7
PL	67.0 ± 6.5	53.6 ± 5.3	50.7 ± 6.4	49.4 ± 6.4	47.6 ± 9.7
ISOK300ext (Nm)					
WI	98.0 ± 6.9	87.2 ± 8.1	89.7 ± 8.1	84.2 ± 8.3	91.5 ± 8.9
WH	101.7 ± 5.6	98.1 ± 6.7	96.3 ± 6.6	91.2 ± 6.8	95.2 ± 7.3
RP	92.2 ± 6.9	87.4 ± 8.1	87.9 ± 8.1	74.4 ± 8.3	90.7 ± 8.9
PL	95.6 ± 6.9	77.5 ± 8.1	85.4 ± 8.1	78.0 ± 8.3	84.2 ± 8.9
ISOK300flex (Nm)					
WI	65.5 ± 4.9	60.5 ± 5.9	57.7 ± 5.4	51.0 ± 4.9	55.3 ± 5.2
WH	63.8 ± 4.0	61.5 ± 4.8	60.3 ± 4.4	60.6 ± 4.0	60.8 ± 4.3
RP	57.5 ± 4.9	53.2 ± 5.9	49.3 ± 5.4	46.0 ± 4.9	48.1 ± 5.2
PL	63.4 ± 4.9	57.4 ± 5.9	52.7 ± 5.4	56.1 ± 4.9	55.5 ± 5.2

4.5 Muscle Soreness

One-way ANOVA indicated that there were no differences between groups at baseline ($p = 0.330$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 4 time points (pre, +24, +48, +72-hrs) indicated no significant group x time interactions ($F_{9, 69} = 0.780$, $p = 0.636$, $\eta_p^2 = 0.092$). There were no significant main group effects ($F_{3, 23} = 1.669$, $p = 0.201$, $\eta_p^2 = 0.179$). There were significant main time effects following the exercise bout ($F_{3, 69} = 49.690$, $p < 0.001$, $\eta_p^2 = 0.684$). Post hoc tests indicated that muscle soreness was increased following the exercise bout and did not return to pre-ECC values at +72-hrs (Figure 2).

4.6 Thigh Circumference

One-way ANOVA indicated that there were no differences between groups at baseline ($p = 0.261$). RMANOVA with 4 groups (WPI, WPH, RPC, PLA) x 4 time points (pre, +24, +48, +72-hrs) indicated there were no group x time effects ($F_{5.463, 41.884} = 1.027$, $p = 0.418$, $\eta_p^2 = 0.118$), main group effects ($F_{3, 23} = 1.452$, $p = 0.254$, $\eta_p^2 = 0.159$), or main time effects ($F_{1.821, 41.884} = 0.880$, $p = 0.413$, $\eta_p^2 = 0.037$) (Figure 3).

CHAPTER 5

DISCUSSION

The present study sought to determine the effects of different sources of pre-sleep protein on muscle recovery in middle aged males following morning exercise. The primary findings of the present study were 1) dairy- and plant-based protein consumed pre-sleep failed to reduce blood markers of inflammation and muscle damage or muscle soreness 2) the eccentric protocol utilized did not induce acute insulin resistance and 3) dairy- and plant-based protein consumed pre-sleep failed to consistently improve muscle function during the 72-hr recovery period. In contrast to our hypotheses, dairy-based proteins were not more effective at improving markers of inflammation and muscle damage, muscle soreness, and muscle function compared to plant-based protein and placebo.

In the present study, CK concentrations increased following exercise and continued to rise, reaching a peak at 72-hrs post-ECC, indicating the eccentric protocol utilized did, in fact, elicit muscle damage. This delayed increase is in line with previous reports, which have indicated peak levels between 3-5 days post-damaging exercise (82, 108, 157). While increases at earlier time points were relatively modest (albeit significant compared to baseline), the increase at 72-hrs post-ECC was a marked increase (414.0 ± 44.9 to 3692.0 ± 457.5 U/L; 791.73% increase from +48-hrs). The present study found no benefit of pre-sleep protein, regardless of source, on CK concentrations. Only 1 other study has examined effects of pre-sleep protein on CK concentrations, which also found no benefit (89). Thus, the present study agrees with Larsen et al. that pre-sleep protein does not attenuate increases of CK following intensive exercise. Others have reported no benefit of protein supplementation on post-exercise CK concentrations (30, 50) which also agrees with the present study. Green et al. observed no differences between placebo, CHO, or CHO+PRO in young, female recreational athletes following downhill running (50). Similarly, Cockburn et al. reported no clear effects of semi-skimmed milk compared to non-caloric placebo following eccentric exercise performed on an isokinetic dynamometer in young, male athletes (30). The present study agrees with Green et al. and Cockburn et al., despite differences in age, gender, training status, and exercise protocols. Differing from the present study, others have reported benefits of protein consumed at other

times on CK concentrations following damaging exercise (17, 31, 54). Brown et al. reported a reduction in CK in active females at 48-hrs post-exercise with whey hydrolysate compared to CHO control, however, no other timepoints were different between treatments during the 72-hr recovery period (17). Hansen et al. reported whey hydrolysate to reduce CK concentrations compared to CHO in young, elite orienteers (men and women) when consumed before and after each exercise session during 1 week of endurance training camp (54). Cockburn et al. reported milk and CHO-PRO beverage reduced CK at 48-hrs compared to CHO only beverage in young, competitive male athletes. In fact, CK did not increase from baseline in either protein group (31). Since age, gender, training status, and exercise protocol cannot explain differences in findings, it is plausible that the timing of protein consumption in relation to the bout of exercise influenced the results. The present study also observed a large variation in CK response to the ECC exercise. Some participants increased less than 1-fold, while others had increases of over 100-fold. High inter-subject variability has been previously reported (10, 77, 124). It has been demonstrated that age (146), training status (110), and body composition (122) are factors that modify muscle damage responses. It is possible that the large variation noted in the present study can be attributed, at least partly, to these factors. The possibility that the total daily protein consumed by our participants was insufficient to elicit favorable attenuations in CK concentrations post-ECC cannot be dismissed.

The muscle damaging exercise protocol utilized in the present study elevated muscle soreness. While the time course of muscle soreness in the present study is similar to the findings of others (63, 116), the present study did not find a benefit of any pre-sleep protein source on reducing exercise induced muscle soreness post-ECC. Previous research has shown a benefit of BCAA (52, 63, 70) and both animal (2, 43) and plant (179) protein supplementation on attenuating exercise induced muscle soreness. Not all studies have shown BCAA (4) or protein (7, 21, 23, 31, 37, 38, 41) supplementation to reduce muscle soreness. Buckley et al. provided 25 g of whey isolate, whey hydrolysate, or a flavored water placebo to young, sedentary men following 100 maximal ECC contractions of the knee extensors of the right leg. While muscle soreness was elevated following ECC, no differences were observed between groups in ratings of muscle soreness in the 24 hour recovery window (21). Similarly, Etheridge et al. provided a milk protein concentrate containing 40 g EAA or a flavored water following a downhill running protocol. In agreement with the present study, muscle soreness was elevated following the

damaging exercise, with no differences between protein or control groups during the 72-hour recovery window (38). Due to differences in timing of protein, it is difficult to make direct translations to the present study. Of the studies providing pre-sleep protein, Abbott et al. reported a large beneficial effect on muscle soreness 12-hrs after a night-time soccer match when 40 g pre-sleep casein protein was ingested compared to iso-energetic CHO (2). Apweiler et al. also provided either 40 g pre-sleep casein or iso-energetic CHO (7). However, in this study, no benefit of pre-sleep protein was reported in men or women following 100 drop jumps performed in the morning (hours away from pre-sleep protein consumption). Similar to Apweiler et al., the present study performed a morning bout of exercise and found no benefit of pre-sleep protein supplementation. Therefore, the most logical explanation for differences in findings is the timing of exercise, whereby pre-sleep protein does not seem to have benefit when exercise is performed in the morning.

IL-6 concentrations were significantly increased at 6-hrs post-ECC in the current study which is in agreement with the notion that muscle damaging exercise results in an immune response (125, 126, 138) and in particular, an increase in IL-6 (70, 91, 121, 127, 179). Protein supplementation has been reported to reduce IL-6 post-exercise (75, 179), which disagrees with the current study. Kerasioti et al. reported a reduction in IL-6 following exercise in younger, physically active men when a carbohydrate-protein supplement was provided (75). It is difficult to translate these to the present study as the present study provided a protein only supplement to middle-aged men. Carbohydrates have been reported to reduce IL-6 following endurance type exercise (112) indicating the carbohydrate provided by Kerasioti et al. could have been a contributing factor to the reductions in IL-6. Xia et al. provided 25 g of oat protein for 14 days prior to a downhill running protocol and for 4 days post-exercise. This group reported IL-6 concentrations to be 29% lower in the oat protein group compared to placebo (179). This study utilized untrained collegiate men, which differs from the recreationally active, middle-aged men utilized in the present study. It is possible that the training status or age of the populations studied could explain the difference in findings as both aging (42, 178) and regular exercise (20, 81) have been reported to alter inflammation. It is also possible that the longer duration of supplementation compared to the present study contributed to the difference in findings. The present study disagrees with the findings of Kerasioti et al. and Xia et al. that protein reduces IL-6 concentrations post-exercise. Not all studies have found benefit of protein on IL-6

concentrations (147). Rowlands et al. reported a likely decrease in IL-6 on day 2 in endurance-trained males when a protein-enriched recovery bar and milk-like drink was consumed compared to energy matched but low protein bar and beverage but possibly trivial overall average effects on IL-6 (147) which agrees with the present study. However, it should be noted that it is difficult to make direct comparisons as no other study to date has examined the effects of pre-sleep protein on IL-6 concentrations during the post-damaging exercise recovery period.

CRP concentrations were significantly greater immediately following exercise compared to baseline which is in line with previous reports showing increases following exercise (19, 75, 97, 154, 179). Brull et al. reported peak CRP concentrations 2-hrs following a 48-hr strenuous military training event in British army recruits (19), which is similar to the findings of the present study. However, Xia et al. reported circulating CRP concentrations to peak at 24-hrs after downhill running and these levels remained elevated from baseline at 96-hrs post-exercise (179), which is not agreement with the current study. There was a large variation in individual response in the present study. The mean difference from baseline to immediately post exercise was 37.92 ± 10.53 ng/mL (5.3 % increase) indicating post-ECC values were greater than baseline. Interestingly, mean differences from 24-hrs post-ECC were 499.19 ± 196.01 ng/mL (69.74% increase). This suggests that while mean concentrations in CRP were actually greatest at 24-hrs, large variability and therefore large standard errors made finding statistically significant differences difficult. To further this point, this large variation in responses can help to explain differences in peak concentrations between studies. Protein supplementation has been shown to attenuate increases in CRP post-exercise (75, 179), which is not in agreement with the present study. Xia et al. showed oat protein to reduce CRP concentrations 72 and 96 hours following a downhill running protocol in young, untrained men (179). The present study did not find protein supplementation to attenuate CRP concentrations compared to placebo. Differences in findings could be attributed to the populations as Xia and group utilized young, untrained men and the present study utilized middle-aged, recreationally active men. Reductions in CRP concentrations have been shown with regular exercise (9). Additionally, Stewart and group reported reduced CRP concentrations following 12 weeks of combined aerobic and resistance training in inactive individuals, but not active individuals (159). Therefore, training status differences between the present study and that of Xia et al. could help explain differences in findings. Further, Xia et al. provided protein supplementation for 14 days prior to the exercise bout, whereas the present

study provided supplementation starting the night of exercise. Therefore, the supplement protocol is another difference which could help explain differences in findings. Other studies have reported no effect of protein supplementation on CRP concentrations during recovery (147). Rowlands et al. reported an overall average unclear effect of protein supplementation in endurance-trained men following 3 high-intensity cycling bouts over 4 days, which agrees with the present study. As Rowlands et al. also used active individuals it is possible that differences in findings can be attributed to training status of the participants. Interestingly, in the present study, when outliers were removed, increases in CRP compared to baseline were not measured. Previous reports have indicated no change in CRP following eccentric exercise (103, 118), however both of these studies utilized the elbow flexors in their protocol. Other studies have shown changes in CRP when utilizing muscle damaging exercise of the knee extensors (97) and prolonged endurance exercise (154).

Following muscle damage, limb swelling has been reported to occur (27, 28, 115, 116, 179). The present study did not find limb swelling to occur post-ECC. Differences in muscle groups examined may help to explain the differences in outcomes. Nosaka et al. utilized maximal eccentric contractions to induce damage of the elbow flexors in resistance training naïve male students. This group reported upper arm circumference to increase post-ECC, with a peak at 3-4 days post-exercise (115). The present study utilized maximal eccentric contractions of the lower limbs in middle-aged males; thus, it is possible that no significant swelling occurring in the current study can be, in part, attributed to the difference in muscle groups and populations studied. Xia et al. utilized a downhill running protocol to induce damage in untrained, college aged males. Thigh girth was increased up to 3 days post-exercise (179). Differences in exercise protocol and population studied may help to explain the differences in findings as the present study utilized recreationally active individuals. However, not all studies have reported swelling following muscle damage (17). Brown et al. implemented a repeated sprint protocol to induce muscle damage in young, trained females. No time effects for thigh or calf girth was reported (17). While the populations studied and protocols used to elicit muscle damage differed, both populations had some training background. Thus, the present study agrees with the findings of Brown et al. that thigh circumference was not increased following damaging exercise when participants were not exercise naïve.

There was no time effect measured for HOMA-IR indicating normal insulin handling post-ECC. This is in contrast to previous reports showing eccentric exercise induced acute insulin resistance (3, 80). Kirwan et al. reported reduced glucose disposal rates during euglycemic-hyperinsulemic clamps following ECC compared to concentric exercise or control at 48-hrs (80), indicating acute insulin resistance following ECC. A later study expanded on ECC induced insulin resistance, showing reduced insulin stimulated IRS1 tyrosine phosphorylation, PI3K activity, and Akt serine phosphorylation following downhill treadmill running compared to control. These molecular alterations were reported in combination with glucose disposal rates that were 19% lower following the ECC versus the non-exercise control trial (3). Kirwan et al. and Del Aguila et al. both used hyperinsulemic clamps to measure glucose disposal rates while the present study utilized HOMA-IR as an indication of insulin resistance. Differences in testing methodologies could explain the non-findings of the present study. Additionally, both studies utilized downhill running protocols while the present study utilized ECC MVC of the knee extensors and flexors. It is also possible that differences in exercise protocol led to differing results. Further, it is possible that the training status of the participants resulted in different findings between studies as Kirwan et al. and Del Aguila et al. utilized untrained participants while the present study utilized recreationally active participants. In support of this notion, Green et al. reported a novel bout of eccentric exercise, specifically downhill treadmill running, to eliminate the increases of glucose and insulin following a second bout of eccentric exercise (51).

As expected, the eccentric protocol utilized in the present study resulted in reduced peak torque in all muscle function variables, indicating damage. Force reduction following intensive exercise has been well reported (2, 7, 18, 29, 150, 160, 174). Pre-sleep protein supplementation has been suggested to attenuate reductions in force production (2, 174); however, the present study did not note benefits of pre-sleep protein on attenuating reductions in isometric or isokinetic peak torque. A pre-sleep bolus of 40 g casein protein resulted in a large benefit of protein consumption on reactive strength index compared to an iso-energetic CHO control in young soccer athletes. This same study reported clear small to moderate benefits of pre-sleep protein compared to CHO on attenuating countermovement jump performance decrements (2). The discrepancy in age of participants between studies could explain differences in findings, though, due to differences in outcome measures, it is difficult to directly translate findings. West et al. reported small to moderate beneficial effects of 25 g whey protein blend compared to CHO

at 10-hr of recovery on peak isometric force of the knee extensors and moderate beneficial effects of protein on the same measure at 24-hr of recovery (174). As the present study also utilized MVC of the knee extensors as an outcome measures, these results are likely more translatable. However, there is still a discrepancy in findings of the benefit of pre-sleep protein between studies. While the age of participants was different in the present study compared to that of both West et al. and Abbott et al., the most likely explanation is the timing of exercise in relation to protein consumption. Both studies utilized night-time exercise, which resulted in pre-sleep protein being ingested in close proximity to the bout of exercise. In contrast, the present study performed exercise in the morning, whereby the pre-sleep protein supplement was consumed hours away from exercise performance. In support of this claim, Apweiler et al. provided a 40 g bolus of pre-sleep protein (the same dose provided in the present study) or iso-energetic CHO to young males and females following a morning bout (0730 – 0900) of 100 drop jumps and reported no benefit of pre-sleep protein on MIVC or CMJ for males, females, or all participants combined (7). The same age discrepancy is present with this study, yet the present study agrees with these findings of no benefit of pre-sleep protein during recovery from damaging exercise and not those of Abbott et al and West et al., which reported benefits of pre-sleep protein on force production during the post-exercise recovery period. Similarly, there was the same difference in force measure, yet, the present study still agrees with Apweiler et al. and not Abbott et al. The difference, again, may be attributed to the exercise timing (morning vs. evening).

Aging has been shown to alter muscle damage (96, 146) and the response to protein feeding (84, 134). Thus, studies examining muscle recovery effects of pre-sleep protein may not be translatable to older populations. Holwerda et al. provided a pre-sleep protein treatment containing 21 g of leucine enriched protein (21 g PRO, 9 g CHO, 3 g fat; 3 g total leucine) compared to an energy matched carbohydrate control (0 g PRO, 25 g CHO, 6 g fat) during 12 weeks of whole-body resistance training in 41 older males. Resistance training was performed 3 times per week between 0800 and 1100. While lean mass, quadriceps cross sectional area, type II fiber cross sectional area, and 1RM strength increased with training, there was no benefit of pre-sleep protein supplementation compared to control (61). It should be noted that previous literature has shown 30 g casein protein nor 30 g casein protein with 2 g of additional leucine to be insufficient to increase MPS during the overnight period, suggesting an optimal dose of 40 g

for this time frame (169). Thus, it is possible that the 21 g of leucine enriched protein was insufficient to notice benefit. However, the present study provided a larger bolus of pre-sleep protein yet did not notice a benefit compared to placebo. It should be noted that the mean age of participants in the present study was 55 ± 7 years, which was younger than that studied by Holwerda and colleagues. Nonetheless, the present study agrees with the findings of Holwerda et al. that pre-sleep protein does not seem to have a benefit for older populations when exercise is performed in the morning hours.

It has been suggested that total daily protein consumption rather than protein timing may be the primary contributor to effects of protein on both muscle size and recovery (73). Following 12 weeks of resistance training, muscle strength, quadriceps cross sectional area, and type II muscle fiber size increased to a greater extent compared to placebo when a protein supplement (27.5 g casein, 15 g carbohydrate) was consumed before sleep (155). However, in this study, the protein group consumed 1.9 ± 0.1 g/kg of protein compared to 1.3 ± 0.1 g/kg. Since groups consumed different total daily protein intakes, it is difficult to attribute the beneficial effects observed solely to the pre-sleep protein supplementation. The present study employed rigorous nutritional control which provided participants with catered meals. These meals were designed to provide 15% of their total daily intake from protein, inclusive of the pre-sleep supplement. This led to a mean intake of 1.08 g/kg, which is above the RDA for daily protein intake. Thus, it is possible that the lack of benefit from pre-sleep protein can be contributed to the daily intake of our participants. It has been suggested that the optimal range for protein intake in adults is 1.2 to 1.6 g/kg (134). While percentage of total daily calories from protein is in range of what adults typically consume (13) and is suggested as the RDA, it is below the suggested optimal range.

However, given the findings of the present study where muscle function was still reduced and soreness and plasma concentrations of CK still elevated at 72-hours, it is likely that the mean intake of 1.08 g/kg was insufficient for recovery in this population altogether. It is now known that the optimal daily protein intake is closer to 1.2 - 1.6 g/kg (134) or 1.4 – 2.0 g/kg (24). In the current study, nearly all muscle function outcomes were still reduced at 72-hours post-ECC, indicating muscle damage and, therefore, lack of recovery. It is possible that we missed the window to see recovery changes in this population, as our participants did not seem to recover fully within 72 hours. It could be that higher daily protein intakes than were provided are required to elicit muscle recovery. The standardized nutrition provided 15% of total calories, as

estimated via indirect calorimetry and adjusted with an activity factor of 1.375, from protein. This is in line with reported intakes for individuals of this age (13, 48). This suggests that the average middle-aged male consumes insufficient protein to elicit recovery benefits for up to 72 hours following intense exercise. Future work should be completed in this population to determine optimal protein intake ranges for muscle recovery. While this is a viable argument for the lack of benefit of pre-sleep protein observed in the present study, it is more likely that the timing of exercise in relation to protein consumption is the primary factor.

Of late, research is beginning to indicate time of training as an important factor for response to pre-sleep protein. Work examining pre-sleep protein utilizing night-time exercise, whereby the protein supplement is consumed in proximity to the training stimulus, has shown benefit (2, 142, 155, 174). West and colleagues examined the effects of 25 g of whey consumed immediately after an evening exercise session (20:00) and again the following morning compared to energy matched carbohydrate supplement. Protein improved 24-hour protein balance compared to placebo. Further, protein showed a moderate beneficial effect for MVC, repetitions to failure, and Wingate peak power (174). Additionally, Abbott and group provided 40 g of casein protein or carbohydrate control, in a crossover design, to young male soccer players the night of a soccer competition (19:00). In the night-time protein group, countermovement jump and reactive strength index decrements were attenuated and muscle soreness was reduced compared to isocaloric carbohydrate during a 60 hour recovery period (2). However, the present study performed the exercise session in the morning, several hours prior to consumption of the pre-sleep protein which differs from West (174) and Abbott (2). Upon further review, it is becoming apparent that utilizing a morning exercise bout rather than night-time exercise reduces or eliminates the effectiveness of pre-sleep protein on muscle recovery (7, 61, 89). Apweiler et al. utilized 100 drop jumps to induce muscle damage in young males and females. A bolus of 40 g casein protein or carbohydrate control was consumed pre-sleep. While reductions in muscular performance following the bout of drop jumps was noted, indicating damage, no benefits were reported for protein consumption in the 48-hours following exercise. This finding was despite the protein group consuming greater total protein than the control group (7). Similarly, Larsen and group provided 0.5 g/kg of either pre-sleep whey protein isolate or carbohydrate to trained male runners for 1 week while performing 11 endurance training sessions performed in the morning and afternoon. Thus, protein supplementation was provided hours after

the training stimulus. Nutritional control was provided by the researchers and protein intake in both groups was set at 1.8 g/kg. No benefit was observed for time trial performance, training volume, or blood biomarkers between groups (89). The present study is in agreement with these studies showing no benefit of pre-sleep protein when protein is consumed hours away from the training stimulus, such as when exercise is performed in the morning. As these studies utilized younger populations, we can extend this concept to middle-aged males. Taken together, these studies support the notion that when exercise is performed in the morning, pre-sleep protein does not seem to have a benefit on muscle recovery or performance. The present study expands upon the current literature by indicating pre-sleep protein, regardless of source, does not have benefit when exercise is performed in the morning. Since prior evening exercise has been shown to augment amino acid incorporation into myofibrillar protein with pre-sleep protein consumption (168), it can be speculated that the lack of benefit with morning exercise can be, in part, attributed to reduced amino acid incorporation into skeletal muscle, though, this has not been examined. Additionally, it has yet to be determined what constitutes night-time exercise. Some athletic events and training sessions take place midday.

5.1 Limitations

The major limitation of the present study is the small sample size. While the intent was to recruit 8 individuals per group, this study was discontinued early in order to preserve the health and safety of participants. To that end, not all 8 participants per group were recruited leaving the sample size underpowered which makes interpretation of the findings difficult. Additionally, procedures were put in place to ensure compliance of all participants to all study procedures. However, as these participants were human, it is not possible to say with 100% certainty that all study procedures were followed while participants were at their homes. Further, the standardized diet contained animal-based protein and was the same for all groups. Thus, individuals in the RP group consumed animal-based proteins as part of their standardized diet which may have altered our findings.

5.2 Conclusions

The major take-aways from this research are that middle-aged men consuming reported average consumption of protein for the age group, which was above the current recommendation for daily protein intake, did not recover 72-hrs following damaging exercise. Pre-sleep protein, regardless of source, did not aid in muscle recovery when damaging exercise was performed in the morning. Intakes of greater than 1.08 g/kg are likely required to promote recovery. Active middle-aged men should consume additional protein, especially if performing exercise bouts which are novel or of unaccustomed intensity. Additionally, it appears that total daily protein consumption is of greater importance than protein timing when exercise is performed in the morning. Taken together, active, middle-aged men should first make sure to consume appropriate per day intakes of protein and to consume a protein meal in close proximity with their exercise sessions to promote muscle recovery. Further, it appears that the protocol used in the present study cannot be deemed safe for this population. While a similar ECC protocol has been cited as being effective at inducing muscle damage (163), it may lead to excessive damage and pursuant rhabdomyolysis in middle-aged men. Caution should be taken when designing muscle damaging protocols in this population.

5.3 Future Directions

Future studies should examine the effects of pre-sleep protein source in middle-aged men utilizing a greater daily intake of protein which more closely matches the optimal intake as described by other groups. Additionally, exercise timing seemingly contributes to the efficacy of pre-sleep protein supplementation, yet no study directly compares morning vs. night-time exercise with pre-sleep protein. Thus, all claims are merely speculative. Future studies should directly compare efficacy of pre-sleep protein with morning vs. night-time exercise. Little is known about what constitutes morning vs evening exercise, or the effects of mid-day training with pre-sleep protein. Future work should examine how much time between exercise stimulus and pre-sleep protein consumption elicits benefits. Additionally, future studies should compare other combinations of plant-based proteins to animal-derived proteins. Future work should examine the effects of plant-based protein combinations on training adaptations over time, both alone and compared to animal-derived proteins. Studies should utilize standardized nutrition meals void of animal-based protein as to determine the effects of consuming a diet void of

animal-based protein (i.e. vegan) compared to a diet with animal-based protein. Lastly, these studies should include enough participants to provide sufficient power to detect differences, should differences exist.

APPENDIX A

IRB APPROVAL LETTER

Florida State
UNIVERSITY

Office of the Vice President For Research
Human Subjects Committee
Tallahassee, Florida 32306-2742
(850) 644-8673 · FAX (850) 644-4392

APPROVAL MEMORANDUM

Date: 01/18/2018

To: Patrick Saracino [redacted]

Address: [redacted]

Dept.: NUTRITION FOOD AND EXERCISE SCIENCES

From: Thomas L. Jacobson, Chair

Re: Use of Human Subjects in Research
The effect of pre-sleep animal or plant-based protein consumption on muscle recovery following damaging exercise in middle-aged men

The application that you submitted to this office in regard to the use of human subjects in the research proposal referenced above has been reviewed by the Human Subjects Committee at its meeting on 12/13/2017
Your project was approved by the Committee.

The Human Subjects Committee has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval does not replace any departmental or other approvals which may be required.

If you submitted a proposed consent form with your application, the approved stamped consent form is attached to this approval notice. Only the stamped version of the consent form may be used in recruiting research subjects.

If the project has not been completed by 12/12/2018 you must request a renewal of approval for continuation of the project. As a courtesy, a renewal notice will be sent to you prior to your expiration date; however, it is your responsibility as the Principal Investigator to timely request renewal of your approval from the Committee.

You are advised that any change in protocol for this project must be reviewed and approved by the Committee prior to implementation of the proposed change in the protocol. A protocol change/amendment form is required to be submitted for approval by the Committee. In addition, federal regulations require that the Principal Investigator promptly report, in writing, any unanticipated problems or adverse events involving risks to research subjects or others.

By copy of this memorandum, the chairman of your department and/or your major professor is reminded that he/she is responsible for being informed concerning research projects involving human subjects in the department, and should review protocols as often as needed to insure that the project is being conducted in compliance with our institution and with DHHS regulations.

This institution has an Assurance on file with the Office for Human Research Protection. The Assurance Number is IRB00000446.

Cc: Michael Ormsbee <mormsbee@fsu.edu>, Advisor
HSC No. 2017.21535

Florida State
UNIVERSITY

Office of the Vice President For Research
Human Subjects Committee
Tallahassee, Florida 32306-2742
(850) 644-8673 · FAX (850) 644-4392

APPROVAL MEMORANDUM

Date: 11/19/2018

To: Patrick Saracino [redacted]

Address: [redacted]

Dept.: NUTRITION FOOD AND EXERCISE SCIENCES

From: Thomas L. Jacobson, Chair

Re: Use of Human Subjects in Research
The effect of pre-sleep animal or plant-based protein consumption on muscle recovery following damaging exercise in middle-aged men

The application that you submitted to this office in regard to the use of human subjects in the research proposal referenced above has been reviewed by the Human Subjects Committee at its meeting on 11/14/2018 Your project was approved by the Committee.

The Human Subjects Committee has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval does not replace any departmental or other approvals which may be required.

If you submitted a proposed consent form with your application, the approved stamped consent form is attached to this approval notice. Only the stamped version of the consent form may be used in recruiting research subjects.

If the project has not been completed by 11/13/2019 you must request a renewal of approval for continuation of the project. As a courtesy, a renewal notice will be sent to you prior to your expiration date; however, it is your responsibility as the Principal Investigator to timely request renewal of your approval from the Committee.

You are advised that any change in protocol for this project must be reviewed and approved by the Committee prior to implementation of the proposed change in the protocol. A protocol change/amendment form is required to be submitted for approval by the Committee. In addition, federal regulations require that the Principal Investigator promptly report, in writing, any unanticipated problems or adverse events involving risks to research subjects or others.

By copy of this memorandum, the chairman of your department and/or your major professor is reminded that he/she is responsible for being informed concerning research projects involving human subjects in the department, and should review protocols as often as needed to insure that the project is being conducted in compliance with our institution and with DHHS regulations.

This institution has an Assurance on file with the Office for Human Research Protection. The Assurance Number is IRB00000446.

Cc:
HSC No. 2018.25794

APPENDIX B

INFORMED CONSENT

Permission to Take Part in a Human Research Study

06/13/2019

IRB Approval Date

Title of research study: ***The effect of pre-sleep animal or plant-based protein consumption on muscle recovery following damaging exercise in middle-aged men***

Investigator: Patrick Saracino, Dr. Michael Ormsbee, Dr. Michael Sweeney

Key Information: The following is a short summary of this study to help you decide whether or not to be a part of this study. More detailed information is listed later on in this form.

Why am I being invited to take part in a research study?

We invite you to take part in a research study to compare the effectiveness of animal- or plant-based pre-sleep protein on muscle damage, metabolism, and performance following eccentric resistance exercise in middle-aged men.

What should I know about a research study?

- Someone will explain this research study to you.
- Whether or not you take part is up to you.
- You can choose not to take part.
- You can agree to take part and later change your mind.
- Your decision will not be held against you.
- You can ask all the questions you want before you decide.

Why is this research being done?

The purpose of the study is to compare the effectiveness of animal- or plant-based pre-sleep protein on muscle damage, metabolism, and performance following eccentric resistance exercise in middle-aged men. Eccentric exercise is defined as exercise where the muscle lengthens under force.

How long will the research last and what will I need to do?

We expect that you will be in this research study for between 2 and 4 weeks (6 visits).

You will be asked to come to the Institute of Sports Sciences and Medicine to complete paperwork, questionnaires, and health history forms. You will also undergo testing for muscle function (power), muscle soreness, inflammation, metabolism, body composition, and sleep quality following a bout of eccentric exercise. You will be asked to consume food provided by the research team for a total of 5 days, as well as a supplement shake on 3 nights.

More detailed information about the study procedures can be found under ***"What happens if I say yes, I want to be in this research?"***

Is there any way being in this study could be bad for me?

Muscle soreness is common from novel exercise or exercise of higher intensity than what is accustomed. However, extreme muscle soreness can lead to hospitalization in some cases. Thus, this research can lead to hospitalization or injury. Infection or bruising is possible during blood sampling. DEXA scans emit small amounts of radiation.

More detailed information about the risks of this study can be found under ***"Is there any way being in this study could be bad for me? (Detailed Risks)"***

Will being in this study help me in any way?

We cannot promise any benefits to you or others from your taking part in this research. However, possible benefits include knowledge about how my body responds to the supplement protocol tested that may improve my muscular recovery and metabolism. With this knowledge I may be able to alter my supplementation or nutritional intakes to translate potential benefits seen in the study into my daily life. Further, I will obtain information regarding power output for selected exercises, which may be helpful in designing future training protocols. Additionally, I will receive information regarding my metabolism.

What happens if I do not want to be in this research?

Participation in research is completely voluntary. You can decide to participate or not to participate.

Your alternative to participating in this research study is to not participate.

Detailed Information: The following is more detailed information about this study in addition to the information listed above.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, talk to the research team at: Patrick Saracino [redacted] or Dr. Michael Ormsbee at [redacted]

This research has been reviewed and approved by an Institutional Review Board ("IRB"). You may talk to them at 850-644-7900 or humansubjects@fsu.edu if:

- Your questions, concerns, or complaints are not being answered by the research team.
- You cannot reach the research team.
- You want to talk to someone besides the research team.
- You have questions about your rights as a research subject.
- You want to get information or provide input about this research.

How many people will be studied?

We expect about 48 people will be in this research study out of 48 people in the entire study nationally

What happens if I say "yes" to being in this research?

If you agree and are eligible to participate in this study, we would ask you to do the following: Come to the Human Performance Laboratory in the Institute of Sports Sciences and Medicine at Florida State University to complete an informed consent document, a health history questionnaire, a physical activity readiness questionnaire, a physical activity questionnaire, an eligibility screening questionnaire, and a 3-day food log. I will also be asked to perform baseline testing for: resting metabolic rate, body composition, and anthropometrics (height and weight), fasted blood-sampling, perceived muscle soreness using a visual analogue scale, thigh circumference, maximal voluntary contraction of the leg muscles, and sleep assessment using fatigue science actigraphy bands (which
will be worn for the remainder of the study). The actigraphy band measures things like movement to indicate sleep quality. It is worn on the wrist like a wristwatch. I will be asked to perform an eccentric exercise protocol on the Biodex dynamometer. The dynamometer is similar to a legs extension and legs flexion exercise machine that measures force at different speeds and angles. Following the eccentric exercise protocol, I will be asked to perform testing for: resting metabolic rate, blood sampling, perceived muscle soreness using a visual analogue scale, thigh circumference, and maximal voluntary contraction. I will be asked to consume a randomized supplement after the exercise session and on three nights: the night of the eccentric exercise and the following two nights. A total of 6 visits will be required: two familiarization trials, eccentric exercise protocol trial, and 3 experimental trials following the eccentric exercise. The experimental trials will be completed within a 3-day period. Each trial will last approximately 1 – 2 hours, with the exception of the exercise day, which will last approximately 8 hours.

On the first visit, I will arrive to the laboratory in the morning between the hours of 0530 and 0830 and complete the informed consent paperwork, medical history questionnaire, physical activity readiness questionnaire, and screening questions.

By meeting these criteria, I will qualify for participation in this study and receive further instruction on details regarding upcoming experimental trials. I will be required to complete a 3-day dietary food and exercise log prior to the experimental trials. These logs should take approximately 1 hour to complete, in total. I will also be instructed to abstain from use of caffeine (12 hours) and alcohol (24 hours) prior to each trial.

My first and second visits to the laboratory will serve as the familiarization to the experimental exercise trials. All details of the familiarization trials are explained below in detail under the experimental trials. I will not receive a treatment supplement during the familiarization trial; however, all other specifications will be exactly the same as the experimental trials.

Over the span of the study, I will be randomly assigned to receive one of the following treatments to be consumed post-eccentric exercise and pre-sleep: (1) 40g whey protein (WP); (2) 40g rice and pea combination protein (RPC); (3) 40g MusclePep whey protein (MP); or (4) Placebo (PL). The supplements will be provided in identical packages and consumed with 350ml water, which will be provided in an opaque bottle.

Visit 1, First familiarization trial, will occur one week prior to the experimental session. I will be asked to arrive at the laboratory in the fasted state between the hours of 0530 and 0830. Height and weight measurements will be taken. I will perform resting metabolic rate testing followed by body composition measurement using BodPod. You will be asked to wear tight fitting, athletic clothing and a cap over your hair while sitting in the BodPod. The testing will take about 5 to 10 minutes. You will be asked to remain seated as still as possible during testing. Risks associated with BodPod testing are extremely minimal. Additionally, as part of your participation in this study, a DEXA scan will be performed. A DEXA is a type of x-ray used to measure bone strength. During this test, x-ray pictures of your body will measure how much fat and muscle are present. You will lie flat on a table and a machine will take pictures of different areas of the body. This test will last about 15 minutes. The results of the DEXA scan for this study will not be shared with you. However, you may request in writing from the principal investigator of this study that the results be sent to your physician. Measurement of total body water will be done using bioelectrical impedance analysis (BIA).

I will then be asked to perform isokinetic (legs extension and legs flexion exercises at a constant speed), eccentric, and isometric (exert force on the machine, but it does not move) maximal voluntary contractions of both limbs. I will perform a submaximal eccentric exercise protocol. I will also be asked to complete a 3-day dietary food log. I will also be asked to wear an actigraphy band to assess sleep and sleep quality for the remainder of the study

Visit 2, Second familiarization trial, will be the same as familiarization trial 1 except body composition measurements will not be performed. All other testing will be the same during the second familiarization trial as the first. The food log will be collected. Food will be provided beginning 2 days prior to the exercise session, and I will be asked to consume only this food during the study as to standardize nutrition. Caloric needs will be calculated using the RMR data and adjusted for activity, which will be determined by an activity questionnaire.

Visit 3, Eccentric exercise protocol experimental trial, will occur the week following the first familiarization. I will arrive to the laboratory in the fasted state between the hours of 0530 and 0830. I will perform resting metabolic rate testing as was performed during familiarization trials. I will then rate my baseline perceived muscle soreness using a visual analogue scale. I will then have baseline thigh circumference measurements taken two times. A blood sample (~10ml) will be collected from the antecubital space of my arm (the crease of the forearm) prior to performing the eccentric exercise protocol. The eccentric exercise protocol will consist of maximal unilateral eccentric knee extension and flexion on both legs for 5 sets of 15 repetitions at 60°/s on an isokinetic dynamometer. Two minutes of rest will be given between sets and 5 minutes between each leg. Following eccentric exercise, a second blood sample will be collected and I will be asked to consume the supplement to which I was assigned, or placebo. This will be consumed within 5 minutes. I will perform maximal voluntary contraction testing on the dominant limb. I will perform resting metabolic rate testing 45 minutes following completion of the exercise protocol. I will then consume the provided standardized meal in the laboratory under supervision of the researchers two hours following completion of the exercise bout (+2 hours). Blood samples will be collected at +4 hours post-exercise and +6 hours post-exercise. I will perform maximal voluntary contraction following the +6 hours blood draw. I will be provided with a supplement packet and an opaque bottle with instructions on how to mix and consume prior to leaving the laboratory. I will record the time of consumption (30 minutes prior to sleep) on the

package after consuming the supplement. I will return the package and bottle to the researchers on each consecutive morning.

Visits 4-6, post-exercise experimental trials, will occur in succession following the eccentric exercise protocol. I will arrive to the laboratory in the fasted state between the hours of 0530 and 0830. I will perform resting metabolic rate testing as was performed during familiarization trials. I will then perform a visual analogue scale to rate post-eccentric exercise perceived muscle soreness. I will perform a thigh circumference measurement on the dominant limb. A blood sample will be collected. Following blood collection, I will perform isokinetic and eccentric maximal voluntary contractions at angular velocities of 60°/s, 180°/s, and 300°/s and isometric maximal voluntary contractions at 60° of extension for both knee extension and flexion. I will be provided with a supplement packet and an opaque bottle with instructions on how to mix and consume prior to leaving the laboratory on visits 4 and 5. I will record the time of consumption (30 minutes prior to sleep) on the package after consuming the supplement. I will return the package and bottle to the researchers on each consecutive morning.

Neither you nor the study doctor will know which treatment you are getting.

What happens if I say "yes," but I change my mind later?

You can leave the research at any time. It will not be held against you.

Is there any way being in this study could be bad for me? (Detailed Risks)

The study has the following risks: I may experience an increased heart rate, sweat rate, muscle soreness, and fatigue related to maximal-effort eccentric exercise testing. Further, I may experience soreness, fatigue, or injury from the maximal-effort voluntary contraction exercise testing, especially if over 60 years of age. All protocols have been previously used in related studies. However, extreme muscle soreness can result in hospitalization, especially in those with sickle cell trait or sickle cell disease. It should be noted that infection and localized bruising and discomfort may be possible during blood sampling. Qualified research personnel will be present during all experimental trials to ensure that proper procedures are followed. All research personnel will be trained in the techniques and use of the equipment during all testing. Individuals trained in blood draws will perform all blood collection.

The risk of injury during the tests will be minimized by careful review of my medical history form. My previous training questionnaire will be analyzed to determine that my current training has prepared me for the caliber of these tests.

Body composition and bone mineral density will be evaluated by DXA that is prescribed and supervised by a medical doctor. This involves low exposure to radiation (less than 5 mREMs per DXA scan). Therefore, subjects undergoing the DXA scan can have a slightly increased cancer risk. By comparison, an x-ray of the spine is 70 mREM and a round trip transcontinental plane flight is 6 mREM.

DEXA scans are commonly performed and considered safe. By participating in this study, you will receive **1** scan. During each scan, you are exposed to a very low amount of radiation. The amount of radiation per scan is less than one tenth of the amount used during a normal chest X-ray and equivalent to one day of exposure to natural background radiation. However, the effects of radiation add up over a lifetime. When deciding to enter this study, think about your past and future contact with radiation. Examples of contact with radiation include x-rays taken for any reason or radiation therapy. The amount of radiation used during a DXA scan is considered safe for adults but has not been determined to be safe for unborn babies. If you are a female of childbearing potential, you will have a pregnancy test prior to performing the DEXA scan.

What happens to the information collected for the research?

Efforts will be made to limit the use and disclosure of your personal information, including research study and medical records, to people who have a need to review this information. We cannot promise complete secrecy. Organizations that may inspect and copy your information include the IRB and other representatives of this organization.

The results of this study may be published but my name or identity will not be revealed. Information obtained during the course of the study will remain confidential to the extent required by law. My name will not appear on any of the results. No individual responses will be reported in publication, only group responses. Confidentiality will be maintained by the assignment of a code number for each subject in which all data recorded will be based. The only record containing both the participant's name and code number will be kept by the principal investigator, Dr. Michael Ormsbee, in a locked drawer in his laboratory. All records will be destroyed after a minimum of five years.

Plasma obtained from blood sampling will be stored in a -80° storage freezer in the Institute of Sports Sciences and Medicine. Samples that are not used upon completion of the study will be

stored for potential use at a future date (up to 7 years). If identifiers are removed from your identifiable private information or identifiable samples that are collected during this research, that information or those samples could be used for future research studies or distributed to another investigator for future research studies without your additional informed consent.

Can I be removed from the research without my OK?

The person in charge of the research study or the sponsor can remove you from the research study without your approval. Possible reasons for removal include becoming aware of a condition which was described as an exclusion criterion.

What else do I need to know?

This research is being funded by Dymatize, Milk Specialties Global, and Puris

If you need medical care because of taking part in this research study, contact the investigator and medical care will be made available. Generally, this care will be billed to you, your insurance, or other third party. Florida State University has no program to pay for medical care for research-related injury.

If you agree to take part in this research study, we will pay you $100 for your time and effort.

Signature Block for Capable

Adult Your signature documents your permission to take part in this research.

Signature of subject | Date

Printed name of subject

Signature of person obtaining consent | Date

06/13/2019

Printed name of person obtaining consent | IRB Approval Date

[Add the following block if a witness will observe the consent process. E.g., short form of consent documentation or illiterate subjects.] My signature below documents that the information in the consent document and any other written information was accurately explained to, and apparently understood by, the subject, and that consent was freely given by the subject.

Signature of witness to consent process | Date

Printed name of person witnessing consent process

APPENDIX C

HEALTH HISTORY QUIESTIONNAIRE

Animal vs. Plant Protein on Muscle Recovery

Health and Exercise Sciences Department and Human Performance Laboratory
[redacted] *Tallahassee, FL 32306*

Date: _____ **ID #:** _____

Health & Fitness History

The purpose of this form is to assess your past and current health and fitness status so that investigators may be aware of any risks or predispositions you have towards injury and disease. Please answer the questions as honestly and as accurately as possible.

1. Have you ever been diagnosed as having any of the following and if yes, how are you currently treating the condition?

Y N High Blood Pressure

Please indicate last known reading:

Blood pressure: _____/_____

Y N High Cholesterol or High Triglycerides

Please indicate last known reading:

Cholesterol: _____

Triglycerides: _____

Y N Diabetes (Circle: Type 1 or Type 2)

Note: Type 1 diabetes is insulin-dependent diabetes mellitus. It is typically diagnosed at an early age and requires insulin shots or an insulin pump immediately upon diagnosis. Type 2 diabetes is often diagnosed at an older age (past age 20) and is usually initially treated with changes in diet and/or medication (pills).

Y N Hypoglycemia (low blood sugar)

Y N Asthma

2. Have you ever had a glucose tolerance test? Y N
If yes, what were the results?

3. Have you ever had a fasting blood sugar test? Y N
If yes, what were the results?

4. Does anyone in your family (immediate family including your grandparents) have a history of cardiovascular disease (heart attacks, stroke, etc.)? Please explain:

5. Do you have any neurological problems including fainting, dizziness, headaches or seizures?

6. Do you have any orthopedic or other health problems that may affect your ability to perform exercise? If yes, please explain:

7. Do you smoke or use smokeless tobacco?

8. Do you drink coffee or other caffeinated beverages? Y N What kind, how much and how often?

9. Please list all vitamins, minerals and herbs and other nutritional supplements as well as medications you are currently taking:

10. Do you have any food allergies or intolerances (e.g., allergic to dairy or lactose intolerance)? Please describe:

11. How would you describe the type of diet you currently eat? Have you recently been on any special diets? What kinds of diets have you used to lose weight or lower cholesterol? Please list and describe:

12. What changes have you made in your diet in the last 6 months?

13. Do you exercise regularly? Y N What kinds of exercise?

How often?

Please describe how much walking you do on a daily basis:

14. How does your current exercise and physical activity compare to 6 months ago? 1 year ago?

15. Have you had a physical exam in the past 2 years? Y N Please describe your assessment of your overall health:

16. Have you ever been diagnosed with sickle cell or sickle cell trait? Y N

APPENDIX D

MUSCLE SORENESS VISUAL ANALOGUE SCALE

Muscle Soreness Visual Analogue Scale

Participant: _____ Trial: _____

Date: _____ Time: _____

On the line below, please make a vertical line indicating your perceived soreness in your **leg muscles**. The left would indicate no soreness at all while the right would indicate extreme soreness, or the worst soreness you have experienced.

0
"No

100
"Extreme Soreness"

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BIOGRAPHICAL SKETCH

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EDUCATION

Doctor of Philosophy, Exercise Physiology, *Anticipated April 2020*
Florida State University, Tallahassee, Florida
Current GPA: 3.581
Major Professor: Dr. Michael Ormsbee

Bachelor of Science, Exercise Science – *Cum Laude, May 2016*
University of North Carolina Wilmington, Wilmington, NC
Minors: Biology, Neuroscience
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RESEARCH

Effects of exercise on Redd1. Gordon BS, Tallahasee, FL

- Specific Laboratory Skills: Western Blot, rtPCR, Cell Culture

Effects of Pre-Sleep Animal or Plant-Based Protein Consumption on Muscle Recovery and Metabolism Following Exercise in Middle-Aged Men. Ormsbee, MJ, Saracino PG, Tallahassee, FL
January 2017 – Current

- Specific laboratory skills: Phlebotomy, centrifuge, pipetting, YSI - Biochemistry Analyzer Enzyme-Linked Immunosorbent Assay (ELISA), Parvo Medics – TrueOne 2400 (RMR), Hologic Discovery Dual Energy X-Ray Absorptiometry (DXA), Biodex isokinetic dynamometer, Visual Analogue Scales, Thigh Circumference, IBM SPSS Statistics 25, GraphPad Prism 8

Anti-Diabetic and Ergogenic Effects of Vitamin D and Resistance Exercise Training on Type 2 Diabetes Mellitus. Kim, J-S, Kim, D-H Tallahassee, FL
October 2017 – December 2017

- Specific laboratory skills: Muscle biopsy, Polymerase Chain Reaction (PCR), Western Blot, pipetting, centrifuge

The Effects of a Caffeine-Like Supplement, Teacrine®, on Muscular Strength and Endurance Performance in Resistance-Trained Men. Ormsbee, MJ Tallahassee, FL
January 2017 – Current

- Specific laboratory skills: Tendo Power Analyzer, 1RM testing

The Effects of Sleep on Inflammation and Performance During the Ultraman Florida Race.
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January 2017-February 2017

- Specific laboratory skills: ELISA, centrifuge, pipetting, VAS questionnaires, Fatigue Science actigraphy bands

Adipose Lipolysis Unchanged by Pre-Exercise Carbohydrate Regardless of Glycemic Index.
Baur, D, Ormsbee MJ, Tallahassee, FL
September 2016 – November 2016

- Specific laboratory skills: Microdialysis, Parvo medics – TrueOne 2400 ($V0_{2max}$), YSI biochemistry analyzer, centrifuge, pipetting

Structure of the Jellyfish Nerve Net. Satterlie, RA, Wilmington, NC
August 2015 – April 2016

- Specific laboratory skills: Immunohistochemistry, thick sectioning of resin blocks, histochemical staining: Toluidine blue, Trichrome, light and fluorescent microscopy

Effects of Static Stretching Vs. Dynamic Warm-Up Protocols on Jump Performance in High School Aged Football Players. Saracino PS. Sprod LK, Wilmington, NC
January 2015 – December 2015

Tracks and Trails, Professor Shields, Wilmington, NC
September 2014, September 2015

PUBLICATIONS

Smith KA, Kisiolek JN, Willingham BD, Morrissey MC, Leyh SM, Saracino PG, Baur DA, Cook MD, Ormsbee MJ. (2019) Ultra-Endurance Triathlon Performance and Markers of Whole-Body and Gut-Specific Inflammation *Eur J Appl Physiol*

Cesareo KR, Mason JR, Saracino PG, Morrissey MC, Ormsbee MJ. (2019) The effects of a caffeine-like supplement, TeaCrine®, on muscular strength, endurance and power performance in resistance-trained men. *J Int Soc Sports Nutr* 16, 47

Saracino PG*, Rosetti LR*, Steiner JL, Gordon BS. (2019) Hormonal Regulation of Core Clock Gene Expression in Skeletal Muscle Following Acute Aerobic Exercise *Biochem Biophys Res Commun 508(3): 871-876*
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Baur DA, **Willingham BD**, **Smith KA**, **Kisiolek JN**, **Morrissey MC**, **Saracino PG**, **Ragland TJ**, **Ormsbee MJ**. **(2018)** Adipose Lipolysis Unchanged by Preexercise Carbohydrate Regardless of Glycemic Index. *Med. Sci. Sport. Exerc.* 50(4): 827-836

REFEREED PRESENTATIONS AT INTERNATIONAL CONFERENCES

Saracino, P. G., Saylor, H. E., Hanna, B. R., Ormsbee, M. J. *Effects of Pre-Sleep Animal vs. Plant-Based Protein Consumption on Inflammation and Muscle Recovery Following Damaging Exercise.* International Sport + Exercise Nutrition Conference, *December 2019*

REFEREED PRESENTATIONS AT CONFERENCES

Saracino, P., Mason, J., Maharaj, A., Salvador, J., Ormsbee, M.J. *Physical Function, Cardiorespiratory Fitness, and Body Composition in Older Individuals*. ACSM National Meeting, Minneapolis, *May 2018*

Kisiolek, J. N., Smith, K. A., Baur, D. A., Willingham, B. D., Morrissey, M. C., Leyh, S. M., Saracino, P. G., & Ormsbee, M. J. *The Effects of Sleep Time on Ultra-Endurance Triathlon Performance*. ACSM National Meeting, Minneapolis, *May 2018*

Smith, K. A., Kisiolek, J. N., Morrissey, M. C., Saracino, P. G., Willingham, B. D., Leyh, S. M., Baur, D. A., & Ormsbee, M. J. *The Effect of Sleep on Systemic Inflammation During the Ultraman Triathlon*. ACSM National Meeting, Minneapolis, *May 2018*

Maharaj, A., Jaime, S., Mason, J., Saracino, P., Figueroa, A. *Skeletal Muscle Oxygenation during Plantarflexion Exercise in Young-Old and Older-Old Adults.* Texas American College of Sports Medicine Annual Conference, March 2018

Saracino, P., Todd, C., Odom, S., & Sprod, L. *Effects of static stretching vs. dynamic warm-up on jump performance of high school aged football players.* Annual Meeting of the Southeast Chapter of the American College of Sports Medicine, *February 2016*

Saracino, P., Todd, C., Odom, S., & Sprod, L. *Effects of static stretching vs. dynamic warm-up on jump performance of high school aged football players.* State of North Carolina Undergraduate Research & Creativity Symposium, *November 2015*

PRESENTATIONS

Effects of Pre-Sleep Animal or Plant-Based Protein Consumption on Muscle Recovery and Metabolism Following Exercise in Middle-Aged Men. Nutrition, Food, and Exercise Sciences Seminar Series, Florida State University, *October 2018*

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Brett Hannah
Hannah Saylor

Undergraduate Students
Kristine Suero
Luis Matamoros
Madison Fraser
Spencer Webb
Ryan Vandenbord
Raegan Mahler, Undergraduate Research Opportunity Program (UROP) *The effects of pre-sleep dairy or plant-based protein consumption on muscle recovery following damaging exercise in middle-aged men*
Emily Hill
Delaney Rahl

TEACHING EXPERIENCE

Teaching Assistant
Exercise Testing and Prescription – PET 4551, undergraduate level
Florida State University, Nutrition, Food, and Exercise Sciences, *January 2018 – May 2018*

- Managed grades on Canvas
- Developed assessment materials
- Developed lecture materials

Teaching Assistant, Instructor of Record
Anatomy and Physiology I Laboratory –PET 3222L, undergraduate level
Florida State University, Nutrition, Food, and Exercise Sciences, *August 2017 – Current*

- Developed course content
- Instructed students
- Created and administered assessments
- Managed gradebooks on Blackboard / Canvas

Teaching Assistant
Intermediary Metabolism of Nutrients II – HUN 3226, undergraduate level
Florida State University, Nutrition, Food, and Exercise Sciences, *January 2017 – May 2017*

- Managed grades on Blackboard
- Created digital forms of exams for online administration through Blackboard
- Created exam questions

Teaching Assistant
Anatomy and Physiology I Laboratory – EXS 216, undergraduate level
University of North Carolina Wilmington, School of Health and Applied Human Sciences,
January 2016 – May 2016

- Designed and edited online assignments
- Designed and instructed review sessions

GUEST LECTURES

Special Topics in Sports Sciences, *Pre-Sleep Protein: Performance Considerations* *2020*

PROFESSIONAL AFFILIATIONS AND HONORS

Omicron Delta Kappa – National Leadership Honor Society, *January 2015 - Current*
Tau Sigma – National Honor Society, *September 2014 – Current*

AWARDS

Outstanding Teaching Assistant Award, Nominee, *February 2018*
School of Health and Applied Human Sciences Outstanding Graduate, *May 2016*

GRANTS AWARDED

Pepper Institute on Aging and Public Policy Conference Travel Grant ($1000) *2019*

Congress of Graduate Students Conference Presentation Support Grant ($500) *2019*

Effects of pre-sleep animal or plant-based protein consumption on inflammatory markers following exercise in middle-aged men,
Dymatize/Milk Specialties Global, *($30,000)* *2018*
Ormsbee, MJ (PI) Saracino, PG (CO-PI)

Graduate Student Advisory Council Presentation Program (Travel Grant) ($325) *2018*

Congress of Graduate Students Conference Presentation Support Grant ($200) *2018*

Effects of pre-sleep animal or plant-based protein consumption on muscle recovery and metabolism following exercise in middle-aged men
Dymatize/Milk Specialties Global, *($31,132.80)* *2018*

Ormsbee, MJ (PI) Saracino, PG (CO-PI)

Effects of static stretching vs. dynamic warm-up protocols on jump performance in high school aged football players
CSURF Undergraduate Research Travel Award ($1,000) *2016*

Effects of static stretching vs. dynamic warm-up protocols on jump performance in high school aged football players.
Ann Sherman Skiba Undergraduate Fellowship ($1,250) *2015*

GRANTS SUBMITTED

GRANTS REJECTED

The effects of pre-sleep dairy or plant-based protein consumption on muscle recovery following damaging exercise in middle-aged women National Dairy Council, $175,615 *2019*
Ormsbee, MJ (PI), Saracino, PG (Co-PI), Fuller SA (Co-PI)

Effects of pre-sleep whole-food vs. supplemental dairy protein on muscle and adipose reconditioning in middle-aged individuals after chronic exercise
National Dairy Council, $318,162 *2019*
Ormsbee, MJ (PI), Fuller SA (Co-PI), Saracino, PG (Co-PI)

The effect of pre-sleep dairy- or plant-based protein consumption on muscle recovery and inflammation following resistance exercise in older men
National Dairy Council, $68,374 *2018*
Ormsbee, MJ (PI) Hickner, RC (Co-PI) Saracino, PG (Co-PI)

Effects of pre-sleep animal or plant-based protein consumption on muscle recovery and metabolism following exercise in middle-aged men,
National Dairy Council, $124,225 *2017*
Ormsbee, MJ (PI) Saracino, PG (Co-PI)

WORKSHOPS ATTENDED

Workshop on Proteomics and Metabolomics, Translational Science Laboratory, College of Medicine, Florida State University *2018*

PROFESSIONAL EXPERIENCE

Strength Coach
Seriously Strong Training, Tallahassee, FL, *January 2017 – May 2017*

- Performed movement analysis testing
- Designed and implemented exercise programming to correct faulty movement patterns and achieve client goals

Internship
Head Strength and Conditioning Coach, UNCW, *January 2016 – May 2016*
Strength Coach
Storm Strength & Fitness, Wilmington, NC, *March 2015 – March 2016*

- Designed and instructed exercise programming for athletes and adults
- Conducted performance testing of athletes from elementary school to D1 Collegiate athletes

Personal Trainer
Gold's Gym, Wilmington, NC, *November 2012 - March 2015*
Louisville Athletic Club, Louisville, KY, *March 2012 – September 2012*

- Designed exercise programming for clients
- Instructed clients on proper execution of exercise movements
- Provided nutritional guidance to clients
- Assisted clients with behavior change to promote a healthier lifestyle

CERTIFICATIONS

Basic Life Support – American Heart Association, *May 2019 – May 2021*
American Registry of Radiologic Technologists – Limited X-ray Machine Operator, *February 2017 – February 2020*
National Strength and Conditioning Association – Certified Strength and Conditioning Specialist, *June 2016 – December 2020*
American College of Sports Medicine – Certified Personal Trainer, *December 2011 - December 2017*
American Red Cross – First Aid, CPR, AED, *December 2012 - April 2018*
USA Weightlifting – Sports Performance Coach, *September 2015 - September 2016*
International Youth Conditioning Association – High School Strength and Conditioning Specialist, *September 2014 - September 2016*
National Exercise and Sports Trainers Association – Sports Injury Specialist, *August 2015* - Lifetime

COMMUNITY AND SERVICE ACTIVITIES

Faculty Search Committee, Exercise Science, *UNCW October 2015 – February 2016*

- Student representative for Faculty Search Committee, Biomechanics professor

Exercise Science Student Association

- Public Relations Officer, *Fall 2015*
- PPD Beach to Battleship Volunteer, *October 2014*
- Organized Adopt-A-Family Volunteer event, *November 2015*

UNCW Barbell Club – President, Founder, *August 2014 - Current*

- Oversee all operations related to the Club
- Maintained active status within the University
- Delegated responsibilities appropriately amongst the active officers to provide a meaningful experience for the active members, now exceeding 50 persons

Alzheimer's Association – Volunteer, *November 2015*

- Help set up and breakdown equipment for the Walk to End Alzheimer's
- Spread awareness and promote funding for Alzheimer's research

Special Olympics Fall Games – Volunteer, *October 2015*

The Miracle League, Wilmington, NC – Volunteer, *March 2015, October 2015*