Pea Protein vs. Whey Protein: Effects of Different Protein Sources on Measures of Oxidative Stress Following Eccentric Exercise

A Thesis
By
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Oxidative stress, an increase in free radicals which can cause damage to macromolecules, has been understudied in regard to resistance exercise, and more specifically eccentric exercise. The purpose of this study was to examine markers of oxidative stress following an eccentric exercise bout in healthy men consuming either whey protein supplementation, pea protein supplementation, or control. The research design utilized a randomized, double-blind approach with untrained, but healthy men. Subjects were randomly assigned to one of three treatment groups: whey protein supplementation, pea protein supplementation, or a eucaloric carbohydrate control (apple juice). Fasting blood samples were collected before subjects completed a 90-minute eccentric exercise bout. Protein supplements were provided at a dose of 0.3 g protein per kg of bodyweight and ingested prior to the exercise bout, immediately post-exercise, and once a day for the following four days. The control group consumed an equivalent caloric dose of apple juice at the same times. Another blood sample was collected immediately post-exercise and subjects reported to the lab at 24, 48, 72, and 96 hours post-exercise for the collection of fasting blood samples. Antioxidant potential via Ferric reducing ability of plasma (FRAP) and oxidative

stress via lipid hydroperoxides (LOOH) were measured in serum samples from all six time points. At the request of the study sponsor, the protein supplements remain blinded, with the codes “Protein A” and “Protein B”. There was no significant interaction found for FRAP ($p=0.831$); however, there was a significant main effect of time ($p = 0.003$). There was significant interaction found for LOOH ($p=0.030$). Post-hoc analyses revealed the change in LOOH pre- to immediately post-exercise was significantly different in the Protein A group ($p = 0.015$). Changes in LOOH from pre- to 72 hours post-exercise was significantly higher in the Protein A group, compared to both the Protein B group ($p = 0.009$) and Apple Juice group ($p = 0.004$). All three treatment groups showed similar increases in antioxidant capacity in response to the eccentric exercise bout, but there was no treatment effect. The different protein supplements did reveal some differences in the pattern of change in lipid hydroperoxides 72 hours after eccentric exercise, but these differences were modest.

Acknowledgments

I would like to thank Dr. Kevin Zwetsloot, Dr. Andrew Shanely and Dr. David Nieman for the support and guidance they have provided me during this project.

Dedication

I would like to dedicate this project to my parents and my sister who taught me the meaning of hard work and how to persevere through challenges and setbacks. I would also like to dedicate this project to Amelia Bruce, who pushed me to be better in every way. Thank you all for your continued support and motivation.

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Introduction

Protein supplements have flooded the market within recent years. Anyone from an elderly patient trying to prevent sarcopenia to world class weightlifters and body builders take them hoping to receive different benefits from supplementation or finding a potential edge. There are many benefits to protein supplementation, most notable are the anabolic effects in skeletal muscle, such as the stimulation of protein synthesis (Witard et al., 2014). It is also well known that skeletal muscle protein synthesis is elevated following a bout of resistance exercise (Rahbek et al., 2014). Furthermore, investigators report additive anabolic effects, increase in strength and hypertrophy, attenuation of muscular weakness following training, and decreased delayed onset of muscle soreness (DOMS) when protein supplementation is combined with a resistance training regimen (Pasiakos, Lieberman, & McLellan, 2014). Protein supplements are often consumed by athletes to decrease soreness and enhance recovery of muscle function and performance by decreasing DOMS (Pasiakos et al., 2014). There is also some evidence indicating that protein supplementation can reduce muscle damage and speed up recovery in resistance trained participants (Buckley et al., 2010; Howatson et al., 2012). There are many different sources of protein that can be used in supplements and researchers do not yet have a consensus on which source of protein is best.

Often referred to as negatives or lengthening contractions, an eccentric contraction is when the muscle is contracting, but the sarcomeres are lengthening, instead of shortening. Eccentric contractions produce higher mechanical forces than concentric contractions, which can lead to muscle damage, soreness, and a loss of muscle function (Buckley et al., 2010). Eccentric exercise also induces higher than normal oxidative stress within the skeletal muscle (Bloomer et al., 2006). Oxidative stress is the result of the production of free radicals in excess of antioxidant

capacity, which can damage proteins and other cellular components, possibly accelerating muscle damage. Free radicals are formed as a product of metabolism and can be produced at higher rates under bouts of elevated stress (Sies, 1997), such as eccentric muscle damage. Free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) cause damage to macromolecules due to their unpaired electron. Cells have evolved antioxidant defense mechanisms, both enzymatic and non-enzymatic, to prevent the accumulation of free radicals and subsequent damage. Antioxidant capacity is a measure of the response against free radicals produced within a biological system. Antioxidant enzymes (e.g., superoxide dismutase) convert free radicals into less reactive molecules, while non-enzymatic antioxidants (e.g., vitamin C) are substances that squelch free radicals by donating an electron to stabilize the ROS or RNS. Both antioxidant defense mechanisms significantly delay or prevent the oxidation of cellular components by preventing the production of ROS/RNS, scavenging ROS/RNS to minimize damage, or repairing damaged molecules (Kunwar & Priyadarsini, 2011).

Many protein supplements on the market contain whey protein, a milk-based protein source containing all the essential amino acids and high concentrations of the branch-chain amino acids (BCAAs): leucine, isoleucine and valine (Craig, 2009). Several studies have reported that whey protein has the ability to attenuate DOMS and increase rate of muscle recovery following exercise (Kim, Lee, & Lee, 2017). Whey protein has also been shown in animal models to attenuate oxidative stress levels, but research in this area in humans is limited in the context of exercise-induced oxidative stress (Athira, Mann, Sharma, & Kumar, 2013). However, with the increasing popularity of vegan diets, it is no surprise that supplement companies are now producing plant-based protein supplement options. These plant-based options, although often containing lower concentrations of BCAAs, have been shown to be a good alternative to whey protein. Babault et al. compared whey protein vs. pea protein with a 12-

week combined resistance training and protein supplementation regimen and reported no difference in the observed increase in muscle thickness between the two protein sources in healthy adult men (Babault et al., 2015). It is unknown whether plant-based protein supplements will elicit similar effects on oxidative stress after resistance exercise as whey protein supplementation (Trapp, Knez, & Sinclair, 2010).

Purpose

The purpose of this study was to examine markers of oxidative stress following an eccentric exercise bout in healthy men consuming either whey protein supplementation, pea protein supplementation, or apple juice, which served as a eucaloric carbohydrate control.

Hypothesis

I hypothesize that the pea protein will be comparable to whey protein as an antioxidant after an eccentric exercise bout. Furthermore, I hypothesize that apple juice will provide similar antioxidant protection to eccentric exercise as the protein supplements.

Literature Review

Eccentric Exercise and Skeletal Muscle Damage

Eccentric resistance exercise produces high mechanical forces leading to muscle damage, soreness, and a loss of muscle function (i.e. strength) (Buckley et al., 2010). The lengthening contractions produce high levels of stress and damage to structural and contractile elements (e.g. myosin and actin) of skeletal muscle that lead to damage and oxidative stress. The possible mechanisms that may cause damage in skeletal muscle from eccentric contractions include free-radical-mediated processes and inflammatory factors within muscle and the junctions between muscles and tendons (Lee et al., 2002). Protein supplements are frequently consumed by athletes in the belief that they will enhance recovery of muscle function and physical performance by attenuating muscle damage and soreness following a previous bout of exercise (Pasiakos et al., 2014). Data suggest that protein supplementation regulates expression of genes involved in repair and remodeling of structural, contractile, and metabolic elements that were damaged by the eccentric contractions (Rowlands et al., 2011). While leucine supplementation has been shown to induce skeletal muscle protein synthesis (Churchward-Venne et al., 2012) and decrease levels of creatine kinase and myoglobin in the blood (markers of muscle damage) following eccentric exercise (Kirby & Triplett, 2012), limited evidence exists whether protein/amino acid supplementation before, during, and following eccentric exercise reduces markers of muscle damage and oxidative stress and accelerates recovery in resistance trained participants (Buckley et al., 2010)(Howatson et al., 2012).

Protein Supplementation

Many studies report an additive anabolic effect of protein/amino acid supplementation after an acute resistance exercise session due to the stimulation of signaling molecules responsible for repair and regeneration of muscle tissue and the contractile elements (Rahbek et al., 2014) (Reitelseder et al., 2011) (Burd et al., 2012). Both mechanical stress and ingestion of amino acids induces the phosphorylation and activation of the signaling molecules mTOR and p70S6kinase, which lead to a positive protein balance, muscle hypertrophy, and increase in strength gains (Rahbek et al., 2014). Longitudinal training studies suggest that increases in strength and muscle mass are greatest when protein (0.25 g high quality protein/kg or 20-40 g) is consumed immediately after exercise.

A meta-analysis of 22 randomized controlled trials and 680 participants showed that post-exercise protein supplementation had a positive effect for fat-free mass (FFM; weighted mean difference of +0.69 kg) and 1-RM leg press strength (weighted mean difference of +13.5 kg), compared with placebo, after a prolonged period of resistance-type exercise training in younger and older participants (Indrio et al., 2016). An increase in FFM or strength gains is often the goal of people who take protein supplements, whether they're athletes, bodybuilders, or someone trying to just get healthier. This meta-analysis extends existing research on the effectiveness of protein supplementation. Even with the best study designs, the effect size for protein supplementation on FFM and strength gains is modest, with high individual variability (Reidy & Rasmussen, 2016). Furthermore, studies suggest no differences between protein types ingested after exercise when a leucine threshold of 700-3000 mg/dose is reached (Reidy & Rasmussen, 2016)(Cambell et al., 2007). The high amount of individual variability means that a specific protein source may lead to significant changes in FFM and strength gains in some individuals, but not others.

The most effective protein source for increases in FFM and strength during resistance training is still under debate. Isotopic tracer studies suggest that whey protein, with high leucine content and its rapid digestibility promotes the greatest anabolic response when ingestion takes place post-exercise. Based on previous BCAA supplementation research, it is assumed that higher amounts of BCAAs are important to reach the leucine threshold, but leucine amounts above that threshold may have diminishing anabolic returns (Reidy & Rasmussen, 2016). While the literature would suggest that consuming protein or free amino acids around resistance exercise can alleviate some aspects of muscle damage, it is still not known whether the source of the protein/amino acids has any influence on markers of muscle damage and oxidative stress (Owens, Twist, Cobley, Howatson, & Close, 2018). Protein supplements increase skeletal muscle protein synthesis post exercise due to an increase in anabolic intracellular signaling, but due to the slow nature of protein turnover following resistance exercise, there are limited effects on acute recovery (Owens et al., 2018). One study reported that whey protein supplementation does attenuate markers of oxidative stress in a rat model of iron-induced oxidative stress, however there needs to be more research done in humans to better determine the effectiveness of protein supplementation on markers of oxidative stress (KIM, PAIK, YOON, & PARK, 2013).

With varying amounts of amino acids found in different foods and protein supplements it is important to investigate the effects of different amino acid profiles. It is believed that the amino acid cysteine possesses antioxidant properties due to its ability to donate a hydrogen from the thiol group (Elias, McClements, & Decker, 2005). Another study investigated antioxidant capacity of the amino acid cysteine against lipid oxidation and found that cysteine derivatives bearing free amino or carboxylate ions possess antioxidant properties as well (Miura, Honda, Masuda, & Masuda, 2014). Methionine, a sulfur-containing amino acid, is also believed to possess antioxidant properties within proteins. Levine et al. found that up to six methionine

residues can be oxidized before ROS/RNS will have substantial negative effects on proteins within the body (Levine, Mosoni, Berlett, & Stadtman, 1996).

Oxidative Stress and Exercise

Exercise-induced oxidative stress has been suggested to contribute to muscle damage following exercise (LEE et al., 2002), such that ROS activity and oxidation of lipids, proteins, glutathione, and DNA can occur from 1 to 4 days post-exercise (Nikolaidis, Jamurtas, & Paschalis, 2008). Catecholamines released under stress during exercise can lead to an increase in ROS/RNS as well, and further production of free radicals can result from prostanoid metabolism, xanthine oxidase, NAD(P)H oxidase and other secondary sources (Urso & Clarkson, 2003). Secondary muscle damage can be caused by changes in blood flow and inflammatory processes in the form of neutrophil and monocyte infiltration (LEE, et al., 2002). Free radicals are neutralized by the body's antioxidant systems, including enzymes, such as superoxide dismutase, catalase, or glutathione peroxidase, as well as non-enzymatic antioxidants, such as vitamins C, A, E or flavonoids (Urso & Clarkson, 2003).

Lipid hydroperoxide (LOOH) detection is used as one measure of oxidative stress, specifically lipid peroxidation assesses membrane phospholipid oxidative stress. Previous studies have found that LOOH levels increase in the blood during and immediately post-exercise in response to both resistance and endurance training, but then trend downward when exercise stops (Çakir-Atabek, Özdemir, & Çolak, 2015). Similarly, LOOH concentrations have been shown to increase systemically during and 1-hour post exercise in response to high intensity resistance training (LEE et al., 2002).

Ferric reducing ability of plasma (FRAP), according to the methods of Benzie (Benzie & Strain, 1996), is used as a measure of the total antioxidant potential of the blood. Resistance

training, especially hypertrophy-inducing training, will increase plasma FRAP transiently following exercise (Hudson et al., 2008), peaking approximately 24 hours post-exercise and returning to baseline approximately 96 hours after exercise (Quindry et al., 2011).

Vegan Diets and Nutritional Limitations

While vegan diets have increased in popularity over the last 20 years (Craig, 2009); however, one of the primary concerns of vegan diets is making sure an individual is consuming the necessary recommended daily amount of proteins and BCAAs. There is little research on the BCAA intake of vegan diets and its effect on skeletal muscle damage/repair mechanisms, let alone the effect on oxidative stress. Current literature shows conflicting results regarding the antioxidant capacity of vegan diets when compared with a traditional omnivore diet and it could be suggested that the large variations in vegan diets contribute to inconsistencies in results (Trapp et al., 2010).

Nutralys® Pea Protein Supplement

The lower concentration of BCAAs contained in many traditional vegan diets may lead to a lower overall nutrient-induced anabolic effect and lower rates of muscle protein synthesis, and subsequent attenuation of hypertrophy/repair mechanisms following exercise. Nutralys® pea protein supplement (Roquette, Lestrem, France), a vegetable protein isolate from the yellow pea (Pisum sativum), contains 85% protein, 7% fat, 3% carbohydrate, and 5% ash. Table 1 compares the amino acid composition for 100 g of Nutralys® and whey protein supplement (Babault et al., 2015). These data demonstrate that the BCAA profile of pea protein (leucine, isoleucine and valine = 14.1 g/100 g) is 22% below that of whey protein (18.1 g/100 g), may be sufficient to support exercise-induced improvements in muscle hypertrophy. Previous research demonstrated that 50 g/day doses of Nutralys® or whey protein during a 12-week resistance training period

resulted in similar increases in muscle thickness, compared to placebo (Babault et al., 2015). As stated previously, leucine supplementation stimulates protein synthesis in skeletal muscle (Churchward-Venne et al., 2012) and decreases markers of muscle damage in the blood following eccentric exercise (Kirby & Triplett, 2012). Nutralys® contains only 74.4% of the leucine of traditional whey protein, leading me to hypothesize that markers of muscle damage and oxidative stress will be higher in the pea protein group, compared to the whey protein group. The best method of protein ingestion after a bout of resistance exercise is unknown, but it has been shown that a single ~20 gram serving of protein provides maximal anabolic stimulus in the early stages of recovery (within 5 hours) (Areta et al., 2013). These findings suggest that the immediate ingestion of protein following the eccentric exercise bout may attenuate oxidative stress at the 24-hour mark.

Table 1. Amino acid composition (g) for 100 g of pea protein or whey protein

	Pea	Whey
Alanine	3.3	4.1
Arginine	6.6	2.1
Aspartic acid	8.9	8.7
Cystine	0.8	1.9
Glutamic acid	13.2	13.9
Glycine	3.1	1.5
Histidine	1.9	1.5
Isoleucine	**3.7**	**4.9**
Leucine	**6.4**	**8.6**
Lysine	5.7	7.2
Methionine	0.8	1.6
Phenylalanine	4.2	2.6
Proline	3.4	4.7
Serine	3.9	4.2
Threonine	2.8	5.7
Tryptophan	0.7	1.5
Tyrosine	3.1	2.8
Valine	**4.0**	**4.6**
Total BCAA:	**14.1**	**18.1**
Total Protein	**76.5**	**82.1**

Methods

Participants

This study and all procedures have been approved by the Appalachian State University Institutional Review Board (IRB #18-0165). The participants for this investigation were recruited from the Kannapolis, NC area from an existing database of people interested in physiology related research. Prior to testing, all participants provided informed consent and filled out a medical release. The sample population included healthy, non-athlete males with some resistance training experience, but not currently engaged in regular resistance training (less than 3 sessions per week), between 18 and 55 years old, with a BMI below 30 (non-obese). Subjects were at "low risk" for cardiovascular disease, as determined by the ACSM screening questionnaire. Volunteers were willing to agree to avoid the use of protein and large-dose vitamin/mineral supplements (above 100% of recommended dietary allowances), herbs, and all medications (in particular, NSAIDs such as ibuprofen and aspirin) during the week of participation. If participants were unable to complete the study requirements, if they had any concurrent conditions which, in the opinion of the primary investigator, would preclude participation or impede with compliance, they were released from the study. Participants were excluded if they had a history of coronary heart disease, stroke, cancer, diabetes, rheumatoid arthritis, high blood pressure, kidney disease, liver disease, blood disease, hormonal disease, or metabolic disease. Potential participants were excluded if they had a history of cancer in the 5 years prior to the screening (except skin cancer that was successfully treated). Participants were excluded if they had any current use of any type of medication that they could not forego for 2 weeks prior to the study. Any participants with a recent history of musculoskeletal trauma (fracture, strain, sprain, etc.), that had not fully healed prior to baseline testing, were excluded from the study.

Research Design

Participants were randomized to either whey protein or Nutralys® pea protein supplement groups or the control group and underwent all study procedures. An apple juice control was selected due to caloric modulation of recovery. The apple juice control is a eucaloric solution so the control subjects were not at a caloric deficit, compared to subjects in the treatment groups. The two protein sources (0.3 g protein/kg body mass) were administered under double-blind procedures, with the codes of "Protein A" and "Protein B" held by the study sponsor, Roquette. At the request of the sponsor, the protein supplements remain blinded until the entire full study and data analyses are completed. Throughout this thesis, the protein supplements will be displayed as "Protein A" and "Protein B", with the investigators unaware of the actual treatment groups. A basic overview of the order of events is outlined in Figure 1 below.

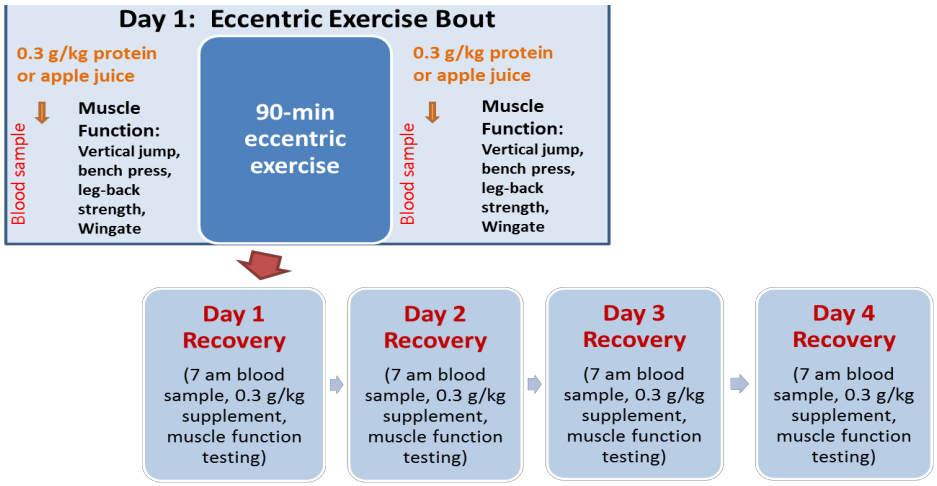

Figure 1: Experimental design. Subjects were randomized into treatment groups, underwent blood sample collection, muscle function tests, and then the 90-minute eccentric exercise bout. Immediately post-exercise there was another blood draw, followed by consumption of treatment and another muscle function test. Subjects came in the next 4 days at the same time for further blood sample collection, muscle function testing, and consumption of supplement.

Familiarization and Screening:

One to two weeks before the study began, participants reported to the ASU Human Performance Lab at the North Carolina Research Campus in Kannapolis, NC in exercise clothes and reviewed and voluntarily signed the informed consent form, completed a screening questionnaire to assess disease risk, and completed a basic demographic/lifestyle questionnaire. Participants were also given an orientation to the eccentric exercise bout and the strength/power performance testing.

Height, body weight, and body composition was measured in a private section of the lab. Bioelectrical impedance (BIA) using the seca BIA scale (seca, Chino, California) was used to assess body composition (i.e., the percent of participant's body weight that is fat tissue) and body weight. Participants removed shoes and socks and stood with bare feet on the BIA scale for about 7-10 seconds while grasping side rails with their hands. Participant's percent body fat was measured via air displacement plethysmography via BodPod (Cosmed, Rome, Italy). Participants sat inside the BodPod with a tight-fitting swim suit for about 10 minutes while their body fat was calculated.

Visit 1:

On day one, participants reported to the lab fasted (no food ingested for the last 12 hours), underwent baseline blood sampling and ingestion of the protein supplements or apple juice, and then completed baseline muscle function testing (described below). Following the baseline testing, participants completed a 90-minute eccentric exercise bout (see Table 2 below). Immediately following the exercise bout, a blood sample was collected and ingestion of a second dose of protein supplements or apple juice, and then completed the second set of muscle function tests. Participants refrained from any food or beverage intake (except for water) for one hour after taking the final supplement dose for the day. Participants returned at 7:00 am in an

overnight fasted state four days in a row after the eccentric exercise bout, on days 2-5 and provided muscle soreness ratings (on a 10-point Likert-scale; 1 = no muscle soreness; 10 = maximal muscle soreness) and blood samples followed by ingestion of the protein supplements or apple juice. Following ingestion of the supplements, participants completed the four muscle function tests. During this week of testing and sample collection, participants engaged in their normal daily diet. Diet logs were not used to monitor diet.

Muscle Function Tests:

1. Vertical jump: Participants will first stand erect with their feet flat on the floor and reach as high as possible with both arms and hands (standing reach height). Participants will then squat down and jump as high as possible with one arm and hand and tap the measuring device on the vertical jump apparatus (jump height; Vertec, West Warwick, Rhode Island). This will be repeated three times, with the best score recorded as the difference between the jump and standing reach heights.

2. Bench press to exhaustion: Participants will lie down supine on a bench, and with spotters standing on either side attempt to bench press a weighted bar equal to 75% of their body weight as many times as possible (to a metronome set at 60 beats/min or 30 lifts/min). The bar must touch a foam block on the chest lightly in the down position, and the arms must be straight in the up position.

3. Leg-Back Strength: Leg/lower back strength will be assessed with a dynamometer. With the legs slightly bent at the knee, participants will grasp a bar attached via a chain and a force measuring device with straight arms, and then lift up with maximal effort for several seconds. This will measure the isometric strength of both legs and back, and will be repeated three times and the highest value will be recorded.

4. 30-Second Wingate Cycling Test: The electronically braked cycle ergometer (Lode Excaliber, Lode B.V., Groningen, Netherlands) will be adjusted to the participant's weight (7 watts per kilogram), and then participants will sprint cycle at maximal speed for 30 seconds. The peak power and average power will be recorded and adjusted to body mass.

Table 2: 90-min Eccentric Exercise Bout

Incline DB Bench	**Utilizing the DB Incline Press, subject completes 3 sets of 5 eccentric reps. Rest for 30-45 secs.**
Bench Press (20 sec)	**Using a 50/95 lb. machine bench, subject completes bench press reps to fatigue in 20 secs then complete 3 eccentric reps with concentric assist. 3 sets total. Rest for 1 minute.**
Lying Med Ball Pass (20 sec)	**Subject assumes supine position with elbows held closely to body and feet on floor with knees bent. Standing overhead, a coach drops an 8/14 lbs. medicine ball, and the subject catches and quickly returns the ball to the coach (20 seconds, 3 sets). Rest for 30 secs.**
Hangs	**Subject assumes neutral handgrip position on pull-up bars. The elbows are flexed to 90° and subject holds this position until fatigue or failure to maintain proper form over 4 sets. Rest for 30 secs.**
Eccentric Lat Pull	**Subject assumes an overhand grip on lat pull-down bar. The bar is pulled down to near chest level and returned to start position with eccentric focus. This is completed for 8 reps, 4 sets. Rest for 1 minute.**
Down Hill Run	**Subject will run on a treadmill at 7mph/8.5mph for 2 minutes at 10% decline for 3 sets. 1 minute rest in between each set.**
Drop Jumps	**Subject will drop from a 12-16" box to the ground and immediately jump vertically for 10 reps, 2 sets. Rest for 30 secs.**
Tuck Jumps	**Subject assumes an athletic stance. On command, the subject will jump vertically for 20 secs (3 sets) in place while emphasizing height and limiting ground contact time. Rest for 30 secs.**
Eccentric Glut-Hamstrings	**With subject in prone position on table, engage in 8 reps, 3 sets of eccentric hamstring curls with each leg. Rest for 30 secs.**
Leg Curls	**With subject in prone position, coach will engage in hamstring curls with towel resistance (8 reps, 2 sets) with each leg. Rest for 30 secs.**
Vertical Skips	**Subject will skip in place as high as they can vertically and land on 1 leg then alternate legs for 30 secs. 3**

	sets. Rest for 1 minute.
Split Squat	**Split leg squats, 15 reps each leg, 3 sets. Rest for 30 secs.**
Shrug Walk	**Subject will walk on treadmill with kettlebell weights in each hand for 0.33 miles, engaging in shoulder shrugs with each step.**
Bottle Shakers	**Subject will assume a seated position with torso leaned slightly backward and feet slightly off ground, and engage in rapid twisting motion with an 8/14 lb. medicine ball for 15 seconds, 3 sets. Rest for 30 secs.**
Crunches	**ACSM abdominal crunches, 20 seconds, 3 sets. Rest for 30 secs.**
Plank	**Prone position on toes and elbows, 45 seconds, 2 sets. Rest for 1 minute.**

Dosing Regimens

As reviewed in the introduction, increases in strength and muscle mass are greatest when protein (0.25 g high quality protein/kg or 20-40 g, target leucine threshold of 700-3000 mg/dose) is consumed immediately after exercise (Pasiakos et al., 2014). A previous study with Nutralys® used a daily dose of 50 g. Given this information, and the study design that had participants reporting to the lab on the day of the eccentric muscle challenge in an overnight fasted state, study participants received two supplement doses (pre- and post-90-min eccentric exercise). The ASU Human Performance Lab successfully employed this same design with NASCAR pit crew athletes when testing a nutritional supplement product for another study sponsor (Nieman et al., 2013). In that same study, an apple juice, no-protein, eucaloric control condition was utilized to ensure that changes in outcome measures over the 4-day study were contrasted statistically in an appropriate fashion. This study will use a dose of 0.3 g per kilogram body weight of protein for both the pea protein and whey protein groups, or 0.3 g per kilogram body weight of apple juice for the control group.

Outcome Measures

For this study, antioxidant capacity via FRAP and oxidative stress via lipid peroxidation (LOOH) in serum samples were used to determine the antioxidant effects of whey protein and the pea protein supplements vs. the apple juice control. Blood samples collected before the first set of muscle function tests and 90-min eccentric exercise bout were used as the baseline control. Blood samples were collected immediately after the 90-min eccentric exercise bout, and again at 24, 48, 72, and 96 hours after the eccentric exercise bout to be analyzed. Post exercise values were compared to the baseline values to assess the antioxidant capacities and oxidative stress levels of the different protein supplements, compared to the apple juice control.

This study used the Ferric Reducing Ability of Plasma (FRAP) and Lipid Hydroperoxide (LOOH) to determine antioxidant potential of different protein sources after eccentric damage. FRAP is considered a highly effective and reproducible test over a wide range of concentrations (Benzie & Strain, 1996). Lipid hydroperoxide measures the amount of oxidized fat tissue found in the blood plasma. Both measures are commonly used as methods that have been validated in many studies investigating oxidative stress.

Antioxidant Capacity via Ferric Reducing Ability of Plasma

The Ferric Reducing Ability of Plasma, or FRAP, assay is one of the gold standard measurements of circulating blood antioxidant capacity. FRAP was measured according to the protocol described by Benzie & Strain (1996). Reagents were prepped first which include 300 mmol/litter acetate buffer, pH 3.6 (3.1 g $C_2H_3NaO_2$ * $3H_2O$ and 16 ml $C_2H_4O_2$ per liter of buffer solution; 10 mmol/liter TPTZ in 40 mmol/liter HCL, 20 mmol/liter $FeCl_3$ * $6H_2O$). Working FRAP reagent was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3$ * $6H_2O$ solution. Samples were prepared using aqueous solutions of known Fe

concentration in the range of 100-1000 μmol/liter, which were used for producing a standard curve. Serum aliquots were thawed on ice as required and used between runs for precision samples.

Oxidative Stress via Lipid Peroxidation

Lipid hydroperoxides, or LOOH, is an efficient method of analyzing lipid peroxidation in an accurate and efficient manner. The assay, like FRAP, quantifies lipid peroxidation by measuring a reaction with ferrous ions. LOOH was analyzed using the protocol supplied by the company providing the assays (Cayman Chemical, Ann Arbor, Michigan). The protocol consists of 1-part sample to be combined with 1-part extract-R and 2 parts cold chloroform (degassed). Once centrifuged, the choloroform extract layer was removed and combined with an equal quantity of chloroform-methanol solution and chromogen. The samples were then added in triplicates to the 96-well plate and absorbances were measured by a spectrophotometer at 500 nm.

Statistical Analysis

The data was analyzed using the Generalized Linear Model, repeated measures ANOVA module in SPSS (IBM, Armonk, New York). The statistical model used utilizes the between participants approach: 3 (groups) x 6 (time points) repeated measures two-way ANOVA with an alpha level set at $p \leq 0.05$. Upon a significant F-ratio, Bonferroni post-hoc analyses were performed to determine differences in specific treatments.

Results

Subjects

Subjects were recruited from the Charlotte, NC metropolitan and surrounding areas. Subjects were untrained, but non-obese and were collected from a database of people interested in physiology-related research. There were no significant differences found between the treatment groups in demographic or anthropometric data (Table 3).

Table 3: Subject Characteristics

	Group A Protein Mean (n=11)	SE	Group B Protein Mean (N=11)	SE	Group C Apple Juice Mean (n=10)	SE
Age	41.09	3.22	41.00	2.77	36.40	3.56
Height (cm)	179.23	2.20	169.40	9.01	177.46	2.44
Weight (kg)	83.95	2.82	80.11	3.09	82.05	4.10
SECA BF (%)	21.03	1.55	21.36	1.78	21.41	1.70
BodPod BF (%)	21.83	1.89	21.21	2.19	22.00	1.96

Table 3: Subject anthropometric data showed no significant difference between any of the 3 groups.

Ferric Reducing Ability of Plasma

Antioxidant capacity, assessed via the FRAP assay, are presented in Figure 2. There was no condition by time interaction found ($p=0.831$). However, there was a significant main effect of time ($p=0.003$), as FRAP values increased over time from baseline (Pre) to 96hr post-exercise. Furthermore, there was no main effect of treatment found.

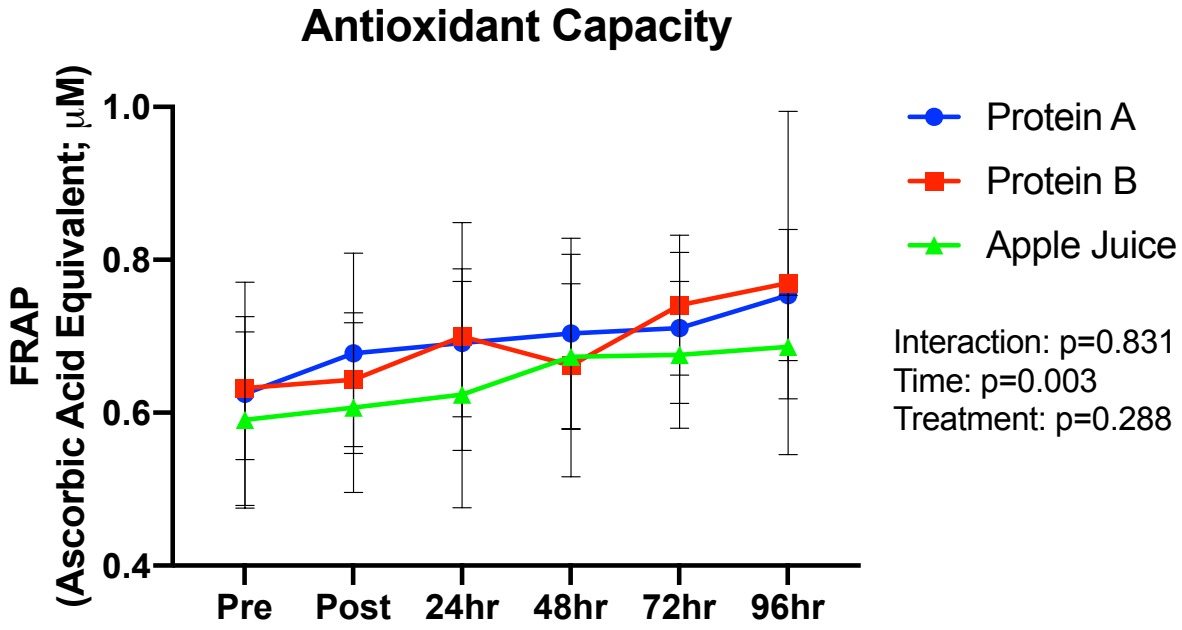

Figure 2: Antioxidant capacity (FRAP) before and in response to an eccentric exercise bout. No treatment x time interaction was found (0.831). No main effect of treatment was found (p=0.288), but there was a significant main effect of time (p = 0.003). Data are presented as Mean ± SD.

Lipid Hydroperoxide

Lipid peroxidation measured via the lipid hydroperoxide assay, is presented in Figure 3. Repeated measures two-way ANOVA discovered a significant time by treatment interaction (p=0.030) in lipid hydroperoxides. Post-hoc analyses revealed that the change in LOOH pre- to immediately post-exercise was significantly different in the Protein A group (p=0.015), compared to the Protein B group, but not the Apple Juice group. Additionally, the change in LOOH from pre- to 72 hours post-exercise was significantly higher in the Protein A group, compared to both the Protein B group (p=0.009) and the Apple Juice group (0.004), however there was no difference between the Protein B and the Apple Juice groups (p=0.184).

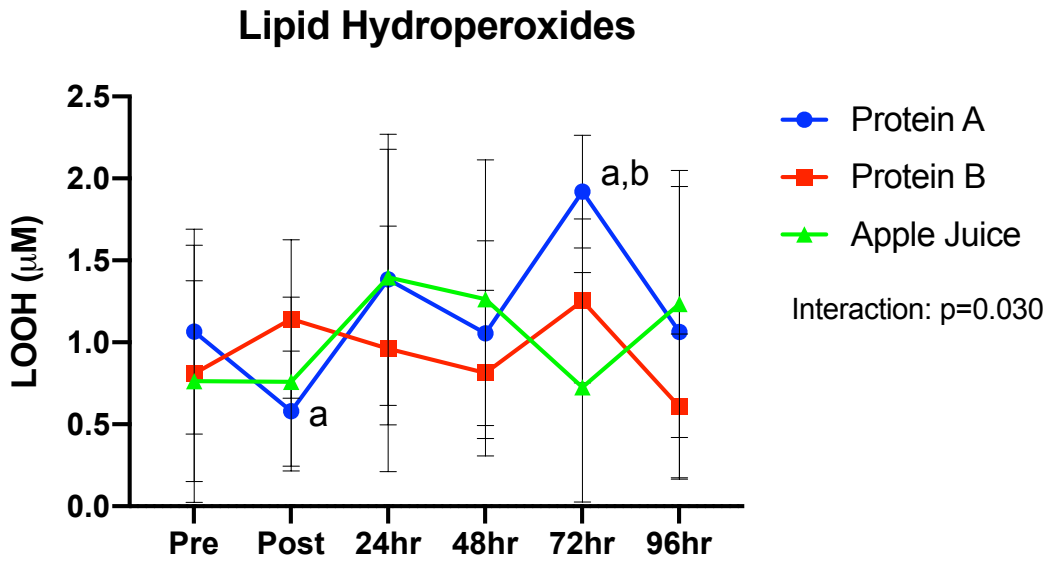

Figure 3: Lipid hydroperoxides before and in response to an eccentric exercise bout. A significant time x group interaction was found (p=0.030). Data are presented as Mean ± SD. a = significant differences between Whey protein and Pea protein. b = Significant differences between Whey protein and Apple juice control.

Discussion

The present study compared the effects of two different protein sources on markers of oxidative stress following a 90-minute eccentric exercise bout. Serum samples were taken at baseline, immediately post-exercise, and at 24, 48, 72, and 96 hours post exercise and analyzed for antioxidant capacity and lipid peroxidation. To our knowledge, this is the first study comparing whey and pea protein supplements on markers of oxidative stress in response to an eccentric exercise bout. The main findings of this investigation were (a) there were no significant differences in antioxidant capacity between the two protein sources; (b) eccentric exercise did cause significant, but variable changes in one marker of oxidative stress, lipid hydroperoxides, over the 5-day testing period.

Exercise-Induced Oxidative Stress*:*

It has been well documented that oxidative stress is induced by prolonged aerobic exercise. In a previous study, older but healthy individuals who underwent low intensity aerobic exercise displayed increased levels of the oxidative stress biomarker malondialdehyde, another measure for lipid peroxidation (Bouzid, Hammouda, Matran, Robin, & Fabre, 2014). Another study compared a squatting protocol with a prolonged cycling protocol to determine differences in acute oxidative stress levels with different training methodologies. This study found no significant changes in malondialdehyde concentrations, however there were significant differences in protein carbonyls (a marker for oxidative damage to proteins) with both exercise mode and time interactions (Bloomer, Goldfarb, Wideman, McKenzie, & Consitt, 2005). Another previous study found that eccentric exercise, in this case downhill running, can cause oxidative stress in women; however they saw no significant oxidative damage to macromolecules, but found subjects had elevated white blood cells, neutrophils, and

lymphocytes following the eccentric exercise bout (Wiecek, Maciejczyk, Szymura, & Szygula, 2017). Furthermore, eccentric exercise consisting of 3 sets of 50 eccentric leg extensions, resulted in no significant effect on LOOH or FRAP; however they did find significant increases in protein carbonyls (Quindry et al., 2011). Findings from our study found that the 90-minute eccentric exercise protocol did not have a detrimental effect on antioxidant capacity. FRAP steadily increased beginning immediately post-exercise and continued to increase until 96 hours post-exercise. These data are consistent with some of the current literature stating that eccentric exercise does not cause a decline in antioxidant capacity in circulation (Hudson et al., 2008). This is further indication that antioxidant capacity doesn't decrease in response to exercise, but instead increases as time post-exercise goes on due to the potential up-regulation of the body's antioxidant defense mechanisms. These data do, however, agree with other studies reporting that antioxidant capacity increases following muscle damaging exercise. The increase in FRAP in this study, along with data found in other studies, could be due to one of several things. Antioxidant capacity could be increasing due to the antioxidant capacities of ingesting the protein and apple juice treatments. Several studies have shown that carbohydrates or carbohydrate-infused beverages can attenuate oxidative stress following exercise (Ferreira et al., 2017). Another possible cause of the increase in antioxidant capacity following exercise is the upregulation of antioxidant defense systems (Ji, 2007).

When oxidative stress increases, the body will try to increase the antioxidant mechanisms to mitigate the increase of free radicals. In our study, LOOH measures did not increase in response to eccentric exercise based on time alone; however, there were variable responses by treatment and time. Hudson et al. (2018) reported that LOOH increases in hypertrophy-based resistance training protocols (lower intensity, higher volume) however they do not increase in strength-based resistance training protocols (high intensity, low volume). These data conflict

with the present study, which included lower intensities with high duration and volume. This is possibly due to differences in rest periods along with treatment effects, such as increased caloric intake modifying metabolic strains due to the treatment being consumed just prior to the start of the 90-minute eccentric exercise bout. One study measured LOOH in response to a resistance training protocol in older adults and found that resistance training attenuates exercise-induced lipid peroxidation (Vincent, Vincent, Braith, Lennon, & Lowenthal, 2002).

Protein and Oxidative Stress*:*

The current literature studying protein supplementation and oxidative stress is very limited in humans. Our findings suggest that protein supplementation may have a beneficial effect on antioxidant capacity. We demonstrate a significant increase in antioxidant capacity (FRAP values) over the study period, which could potentially be an effect from days of protein and carbohydrate supplementation after eccentric exercise that may help attenuate oxidative stress. This is in agreement with one study that examined antioxidant capacity and lipid peroxidation in iron-overload induced oxidative stress in rats, which found that TRAP (an antioxidant capacity measure) increased significantly in rats that received whey protein supplementation, compared with rats that received no protein supplementation. This study also found that conjugated dienes (measure of lipid peroxidation) was attenuated which is not what our data suggests based on supplementation alone, however whether or not this applies to exercise-induced oxidative stress is unknown (Kobayashi et al., 2013). Furthermore, there is evidence showing that whey protein can increase plasma biomarkers of antioxidant capacity, both in response to illness or high intensity exercise (Corrochano, Buckin, Kelly, & Giblin, 2018). Whey protein has also been shown to significantly lower malondialdehyde concentrations, as well as increase glutathione peroxidase, cellular superoxide dismutase, and catalase activity in human lung fibroblasts *in vitro* (Kong, Peng, Xiong, & Zhao, 2012). Taken

together, these findings provide insight as to why FRAP values increased and why there were no consistent changes in the pattern of LOOH in response to eccentric exercise with the different treatment groups in our study. One of the common questions regarding protein sources, including whey protein, is whether or not these sources elicit lasting and significant effects in the human body, not just in cell-culture models. One study addressed this question by simulating the harsh environment of the GI tract and found that bovine-derived protein sources, such as whey protein, provide antioxidant defenses following digestion (Corrochano, Sariçay, et al., 2018). Although there is some evidence that protein supplementation may attenuate oxidative stress, a recent systematic review found that protein supplementation is still not as effective as other known nutrients, such as carbohydrate or green tea extract, in reducing oxidative stress. Further research is needed to determine if protein supplementation provides antioxidant protection, independent of exercise.

Non-Protein Nutritional Interventions on Oxidative Stress

While the effect of protein supplementation on oxidative stress remains relatively unclear, there are other examples of antioxidant capacity changes in response to non-protein nutritional interventions. The present study showed no significant differences in overall antioxidant capacity between the two protein supplements and the apple juice control. It was initially hypothesized that the protein groups would have overall higher antioxidant capacity but that isn't what our findings suggested. Apple juice, which contains a large quantity of carbohydrates, also increased antioxidant capacity equivalent to the protein supplements. This is consistent with many previous studies suggesting that carbohydrate supplementation elicit antioxidant protection from exercise-induced oxidative stress. A recent study found that acute carbohydrate supplementation attenuated post-exercise plasma levels of cytochrome P450-generated oxylipins, which are bioactive oxidation products (Nieman et al., 2019). This study, as

well as others in the literature, may provide an explanation into why there were no significant differences between the protein treatments and the apple juice control in the current study.

Plant- vs. Animal-Derived Protein Sources

The main objective of this study was to investigate whether plant-based protein supplementation elicits comparable bioactive effects to those of animal-based protein supplements. Past research has demonstrated that while both plant- and animal-based protein sources possess anabolic properties, plant-based proteins induce less anabolic effects and potentially less muscle hypertrophy, compared to animal-based protein sources (Vliet & Burd, 2015). While this study did not investigate anabolic properties, our data suggests that there are no significant differences between the two protein supplement treatments on markers of oxidative stress. It is plausible that these two sources of protein, with potential differences in amino acid composition, digestion, and amino acid absorption kinetics, could still render different biological effects in areas other than oxidative stress.

Amino acids are important for many mechanisms of recovery and regeneration of tissue following muscle damaging exercise. I hypothesized that these two protein sources would have a large effect on attenuating oxidative stress. Research has shown that BCAA supplementation can upregulate SOD and SOD2 antioxidant mechanisms and play an important role in a pathological model of liver oxidative stress (Kobayashi et al., 2013). Our findings are in agreement with Kobayashi et al. (2013), however because there are no significant differences between our protein groups, we can't conclude that the differing concentrations of amino acids play a role in exercise-induced oxidative stress. BCAA supplementation has also been shown to increase performance and antioxidant capacity in both sedentary and trained animal models (Pop, Mureşan, & Bondor, 2009). Cysteine and methionine are two sulfur-based amino acids that play

important roles in antioxidant defense. Whey protein contains higher levels of cysteine and methionine, compared to the pea protein. Diets low in sulfur-based amino acids have been shown to increase oxidative stress and a decrease in glutathione expression (Kusama-Eguchi et al., 2011). Cysteine and methionine concentrations and their relation to oxidative stress is an understudied area that shows promise for increasing oxidative stress both in pathology and exercise-induced oxidative stress models.

Limitations

A major limitation in this study was the lack of a non-nutritive control group. As demonstrated previously, there is a large body of literature that shows carbohydrate supplementation provides significant protection against exercise-induced oxidative stress. The current study used apple juice as a eucaloric control; however apple juice contains not only carbohydrates, but also other potentially bioactive compounds, such as polyphenols and phytochemicals (insert ref PMID: 18855307), that could attenuate oxidative stress. While this study does reveal some interesting findings comparing two different sources of protein on oxidative stress mechanisms, we exercise caution in drawing firm conclusions regarding the effectiveness of either pea protein or whey protein supplementation on attenuating exercise-induced oxidative stress. Future studies should include a non-nutritive control group, such as water only, to serve as an effective control. A water only control group would have likely revealed that participants receiving only water after the eccentric exercise protocol may experience a very high level of oxidative stress, compared to the protein and apple juice treatment groups.

References

Areta, J. L., Burke, L. M., Ross, M. L., Camera, D. M., West, D. W. D., Broad, E. M., … Coffey, V. G. (2013). Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *Journal of Physiology*, *591*(9), 2319–2331. doi.org/10.1113/jphysiol.2012.244897

Athira, S., Mann, B., Sharma, R., & Kumar, R. (2013). Ameliorative potential of whey protein hydrolysate against paracetamol-induced oxidative stress. *Journal of Dairy Science*, *96*(3), 1431–1437. doi.org/10.3168/jds.2012-6080

Babault, N., Christos Païzis, Deley, G., Laetitia Guérin-Deremaux, Marie-Hélène Saniez, Lefranc-Millot, C., & Allaert, F. A. (2015). Pea proteins oral supplementation promotes muscle thickness gains during resistance training: A double-blind, randomized, Placebo-controlled clinical trial vs. Whey protein. *Journal of the International Society of Sports Nutrition*, *12*(1). doi.org/10.1186/s12970-014-0064-5

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76. doi.org/10.1006/abio.1996.0292

Bloomer, R. J., Falvo, M. J., Fry, A. C., Schilling, B. K., Smith, W. A., & Moore, C. A. (2006). Oxidative stress response in trained men following repeated squats or sprints. *Medicine and Science in Sports and Exercise*, *38*(8), 1436–1442. doi.org/10.1249/01.mss.0000227408.91474.77

Bloomer, R. J., Goldfarb, A. H., Wideman, L., McKenzie, M. J., & Consitt, L. A. (2005). Effects of Acute Aerobic and Anaerobic Exercise on Blood Markers of Oxidative Stress. *The Journal of Strength and Conditioning Research*, *19*(2), 276. doi.org/10.1519/14823.1

Bouzid, M. A., Hammouda, O., Matran, R., Robin, S., & Fabre, C. (2014). Low intensity aerobic exercise and oxidative stress markers in older adults. *Journal of Aging and Physical Activity*, *22*(4), 536–542. doi.org/10.1123/JAPA.2013-0037

Buckley, J. D., Thomson, R. L., Coates, A. M., Howe, P. R. C., DeNichilo, M. O., & Rowney, M. K. (2010). Supplementation with a whey protein hydrolysate enhances recovery of muscle force-generating capacity following eccentric exercise. *Journal of Science and Medicine in Sport*, *13*(1), 178–181. doi.org/10.1016/j.jsams.2008.06.007

Burd, N. A., Yang, Y., Moore, D. R., Tang, J. E., Tarnopolsky, M. A., & Phillips, S. M. (2012). Greater stimulation of myofibrillar protein synthesis with ingestion of whey protein isolate v. micellar casein at rest and after resistance exercise in elderly men. *British Journal of Nutrition*, *108*(6), 958–962. doi.org/10.1017/S0007114511006271

Çakir-Atabek, H., Özdemir, F., & Çolak, R. (2015). Oxidative stress and antioxidant responses to progressive resistance exercise intensity in trained and untrained males. *Biology of Sport*, *32*(4), 321–328. doi.org/10.5604/20831862.1176302

Cambell, B., Kreider, R. B., Ziegenfuss, T., Bounty, P. La, Roberts, M., Burke, D., … Antonio, J. (2007). International Society of Sports Nutrition position stand: protein and exercise. *Journal of International Society of Sports Nutrition*, *4*(23), 1–5. doi.org/10.1186/1550-2783-4-Received

Churchward-Venne, T. A., Burd, N. A., Mitchell, C. J., West, D. W. D., Philp, A., Marcotte, G. R., … Phillips, S. M. (2012). Supplementation of a suboptimal protein dose with leucine or essential amino acids: Effects on myofibrillar protein synthesis at rest and following resistance exercise in men. *Journal of Physiology*, *590*(11), 2751–2765. doi.org/10.1113/jphysiol.2012.228833

Corrochano, A. R., Buckin, V., Kelly, P. M., & Giblin, L. (2018). Invited review: Whey proteins as antioxidants and promoters of cellular antioxidant pathways. *Journal of Dairy Science*, *101*(6), 4747–4761. doi.org/10.3168/jds.2017-13618

Corrochano, A. R., Sariçay, Y., Arranz, E., Kelly, P. M., Buckin, V., & Giblin, L. (2018). Comparison of antioxidant activities of bovine whey proteins before and after simulated gastrointestinal digestion. *Journal of Dairy Science*, *102*(1), 54–67. doi.org/10.3168/jds.2018-14581

Craig, W. (2009). Health effeets of vegan diets 1-3. *The American Journal Of Clinical Nutrition*, *89*(April), 1627–1633. doi.org/10.3945/ajcn.2009.26736N.Am

Elias, R. J., McClements, D. J., & Decker, E. A. (2005). Antioxidant activity of cysteine, tryptophan, and methionine residues in continuous phase β-lactoglobulin in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, *53*(26), 10248–10253. doi.org/10.1021/jf0521698

Ferreira, D. D. S., Toscano, L. T., Rocha, L. L. L., Cerqueira, G. S., De França, A. C. L., Dos Santos, M. A. P., & Silva, A. S. (2017). Carbohydrates plus protein reduces oxidative stress after single bout of aerobic exercise. *Journal of Human Sport and Exercise*, *12*(4), 1208–1219. doi.org/10.14198/jhse.2017.124.07

Goldfarb, A. H., Bloomer, R. J., & Mckenzie, M. J. (2005). Combined antioxidant treatment effects on blood oxidative stress after eccentric exercise. *Medicine and Science in Sports and Exercise*, *37*(2), 234–239. doi.org/10.1249/01.MSS.0000152887.87785.BE

Howatson, G., Hoad, M., Goodall, S., Tallent, J., Bell, P. G., & French, D. N. (2012). Exercise-induced muscle damage is reduced in resistance-trained males by branched chain amino acids: a randomized, double-blind, placebo controlled study. *Journal of the International*

Society of Sports Nutrition, *9*, 20. doi.org/10.1186/1550-2783-9-20

Hudson, M. B., Hosick, P. A., Mccaulley, G. O., Schrieber, L., Wrieden, J., Mcanulty, S. R., … Quindry, J. C. (2008). The effect of resistance exercise on humoral markers of oxidative stress. *Medicine and Science in Sports and Exercise*, *40*(3), 542–548. doi.org/10.1249/MSS.0b013e31815daf89

Indrio, F., Riezzo, G., Raimondi, F., Di Mauro, A., Francavilla, R., Elena, M., … Malik, A. (2016). Hydrogen breath tests in gastrointestinal diseases. *Good Nutrition: Perspectives for the 21st Century*, *29*(4), 31–44. doi.org/10.3945/ajcn.112.037556.INTRODUCTION

Ji, L. L. (2007). Antioxidant signaling in skeletal muscle: A brief review. *Experimental Gerontology*, *42*(7), 582–593. doi.org/10.1016/j.exger.2007.03.002

Karatas, F., Karatepe, M., & Baysar, a. (2002). Determination of free malondialdehyde in human serum by high-performance liquid chromatography. *Analytical Biochemistry*, *311*(1), 76–79. doi.org/10.1016/S0003-2697(02)00387-1

Kim, J., Lee, C., & Lee, J. (2017). Effect of timing of whey protein supplement on muscle damage markers after eccentric exercise. *Journal of Exercise Rehabilitation, 13*(4), 436–440. doi.ort/10.12965/jer.1735034.517

Kim, J., Paik, H.-D., Yoon, Y.-C., & Park, E. (2013). Whey Protein Inhibits Iron Overload-Induced Oxidative Stress in Rats. *Journal of Nutritional Science and Vitaminology*, *59*(3), 198–205. doi.org/10.3177/jnsv.59.198

Kirby, T. J., & Triplett, N. T. (2012). Effect of leucine supplementation on indices of muscle damage following drop jumps and resistance exercise. *Amino Acids,* 1987–1996. doi.org/10.1007/s00726-011-0928-9

Kobayashi, Y., Takei, Y., Sugimoto, R., Iwasa, M., Fujita, N., Hara, N., … Mifuji-Moroka, R. (2013). Branched-Chain Amino Acid Supplementation Reduces Oxidative Stress and Prolongs Survival in Rats with Advanced Liver Cirrhosis. *PLoS ONE*, *8*(7), e70309. doi.org/10.1371/journal.pone.0070309

Kong, B., Peng, X., Xiong, Y. L., & Zhao, X. (2012). Protection of lung fibroblast MRC-5 cells against hydrogen peroxide-induced oxidative damage by 0.1-2.8 kDa antioxidative peptides isolated from whey protein hydrolysate. *Food Chemistry*, *135*(2), 540–547. doi.org/10.1016/j.foodchem.2012.04.122

Kunwar, A., & Priyadarsini, K. . (2011). Free radicals, oxidative stress and importance of antioxidants in human health. *Journal of Medical and Allied Sciences*, *1*(2), 53–60. doi.org/10.1007/s11746-998-0032-9

Kusama-Eguchi, K., Yoshino, N., Minoura, A., Watanabe, K., Kusama, T., Lambein, F., & Ikegami, F. (2011). Sulfur amino acids deficiency caused by grass pea diet plays an important role in the toxicity of l-β-ODAP by increasing the oxidative stress: Studies on a motor neuron cell line. *Food and Chemical Toxicology*, *49*(3), 636–643. doi.org/10.1016/j.fct.2010.07.049

Lee, J., Goldfarb, A. H., Rescino, M. H., Hegde, S., Patrick, S., & Apperson, K. (2002). Eccentric exercise effect on blood oxidative-stress markers and delayed onset of muscle soreness. *Medicine and Science in Sports and Exercise*, *34*(3), 443–448. doi.org/10.1097/00005768-200203000-00010

Lee, J., Goldfarb, A., Rescino, M., Hedge, S., Patrick, S., & Apperson, K. (2002). Eccentric exercise effect on blood oxidative-stress markers and delayed onset of muscle soreness. *Medicine & Science in Sports & Exercise March 2002*, *34*(3), 443–448.

doi.org/10.1097/00005768-200203000-00010

Levine, R. L., Mosoni, L., Berlett, B. S., & Stadtman, E. R. (1996). Methionine residues as endogenous antioxidants in proteins. *Proceedings of the National Academy of Sciences*, *93*(26), 15036–15040. doi.org/10.1073/pnas.93.26.15036

Miura, Y., Honda, S., Masuda, A., & Masuda, T. (2014). Antioxidant activities of cysteine derivatives against lipid oxidation in anhydrous media. *Bioscience, Biotechnology and Biochemistry*, *78*(8), 1452–1455. doi.org/10.1080/09168451.2014.918496

Nieman, D. C., Gillitt, N. D., Andrew Shanely, R., Dew, D., Meaney, M. P., & Luo, B. (2013). Vitamin D2supplementation amplifies eccentric exercise-induced muscle damage in NASCAR pit crew athletes. *Nutrients*, *6*(1), 63–75. doi.org/10.3390/nu6010063

Nieman, D. C., Gillitt, N. D., Chen, G.-Y., Zhang, Q., Sakaguchi, C. A., & Stephan, E. H. (2019). Carbohydrate intake attenuates post-exercise plasma levels of cytochrome P450-generated oxylipins. *Plos One*, *14*(3), e0213676. doi.org/10.1371/journal.pone.0213676

Nikolaidis, M. G., Jamurtas, A. Z., & Paschalis, V. (2008). The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress. *Sports Medicine*, *38*(7), 579–606. Retrieved from http://link.springer.com/article/10.2165/00007256-200838070-00005. doi.org/ https://dx.doi.org/10.2165/00007256-200838070-00005

Owens, D. J., Twist, C., Cobley, J. N., Howatson, G., & Close, G. L. (2018). Exercise-induced muscle damage: What is it, what causes it and what are the nutritional solutions? *European Journal of Sport Science*, 19(0), 1–15. doi.org/10.1080/17461391.2018.1505957

Pasiakos, S. M., Lieberman, H. R., & McLellan, T. M. (2014). Effects of protein supplements on muscle damage, soreness and recovery of muscle function and physical performance: A

systematic review. *Sports Medicine*, *44*(5), 655–670. doi.org/10.1007/s40279-013-0137-7

Pop, N. H., Mureşan, A., & Bondor, C. (2009). Efectul suplimentării cu fosfocreatină asupra balanţei oxidanţi / antioxidanţi în efort The effect of phosphocreatine supplementation on the oxidants / antioxidants balance during physical exercise. *Science & Sports*, *3*(3), 285–289. Search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=120285859&site=eds-live&scope=site

Quindry, J., Miller, L., McGinnis, G., Irwin, M., Dumke, C., Magal, M., … Urbiztondo, Z. (2011). Muscle-fiber type and blood oxidative stress after eccentric exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, *21*(6), 462–470. doi.org/10.1123/ijsnem.21.6.462

Rahbek, S. K., Farup, J., Møller, A. B., Vendelbo, M. H., Holm, L., Jessen, N., & Vissing, K. (2014). Effects of divergent resistance exercise contraction mode and dietary supplementation type on anabolic signalling, muscle protein synthesis and muscle hypertrophy. *Amino Acids*, *46*(10), 2377–2392. doi.org/10.1007/s00726-014-1792-1

Reidy, P. T., & Rasmussen, B. B. (2016). Role of Ingested Amino Acids and Protein in the Promotion of Resistance Exercise-Induced Muscle Protein Anabolism. *Journal of Nutrition*, *146*(2), 155–183. doi.org/10.3945/jn.114.203208

Reitelseder, S., Agergaard, J., Doessing, S., Helmark, I. C., Lund, P., Kristensen, N. B., … Holm, L. (2011). Whey and casein labeled with L - [1- 13 C] leucine and muscle protein synthesis : effect of resistance exercise and protein ingestion. *American Journal of Physiology, Endocrinology, and Metabolism*, *300*, E231–E242. doi.org/10.1152/ajpendo.00513.2010.

Rowlands, D. S., Thomson, J. S., Timmons, B. W., Raymond, F., Fuerholz, A., Mansourian, R.,

… Mc, Z. (2011). Transcriptome and translational signaling following endurance exercise in trained skeletal muscle: impact of dietary protein. *Physiological Genomics*, *43*(17), 1004–1020. doi.org/10.1152/physiolgenomics.00073.2011

Sies, H. (1997). Oxidative stress: Oxidants and antioxidants. *Experimental Physiology*, *82*(2), 291–295. doi.org/10.1113/expphysiol.1997.sp004024

Trapp, D., Knez, W., & Sinclair, W. (2010). Could a vegetarian diet reduce exercise-induced oxidative stress? A review of the literature. *Journal of Sports Sciences*, *28*(12), 1261–1268. doi.org/10.1080/02640414.2010.507676

Urso, M. L., & Clarkson, P. M. (2003). Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*, *189*(1–2), 41–54. doi.org/10.1016/S0300-483X(03)00151-3

Vincent, K. R., Vincent, H. K., Braith, R. W., Lennon, S. L., & Lowenthal, D. T. (2002). Resistance exercise training attenuates exercise-induced lipid peroxidation in the elderly. *European Journal of Applied Physiology*, *87*(4–5), 416–423. doi.org/10.1007/s00421-002-0640-2

Vliet, S. Van, & Burd, N. A. (2015). The Skeletal Muscle Anabolic Response to Plant- versus. *Journal of Nutrition,* 145(9), 1981-1991. doi.org/10.3945/jn.114.204305.

Wiecek, M., Maciejczyk, M., Szymura, J., & Szygula, Z. (2017). Sex differences in oxidative stress after eccentric and concentric exercise. *Redox Report*, *22*(6), 478–485. doi.org/10.1080/13510002.2017.1304195

Witard, O. C., Jackman, S. R., Breen, L., Smith, K., Selby, A., & Tipton, K. D. (2014). Myofibrillar muscle protein synthesis rats subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *American Journal of Clinical*

Nutrition, *99*(April), 86–95. doi.org/10.3945/ajcn.112.055517

Vita

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