Skeletal muscle damage, delayed onset muscle soreness and performance after resistance training with leucine and carbohydrate or carbohydrate alone

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SKELETAL MUSCLE DAMAGE, DELAYED ONSET MUSCLE
SORENESS AND PERFORMANCE AFTER RESISTANCE
TRAINING WITH LEUCINE AND CARBOHYDRATE
OR CARBOHYDRATE ALONE

by

Matthew Steven Stock
Bachelor of Science
Florida Atlantic University
2006

A thesis submitted in partial fulfillment
of the requirements for the

Masters of Science Degree in Kinesiology
Department of Kinesiology
College of Allied Health Sciences

Graduate College
University of Nevada, Las Vegas
August 2008
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Entitled
Skeletal Muscle Damage, Delayed Onset Muscle Soreness, and Performance
after Resistance Training with Leucine and Carbohydrate or Carbohydrate
Alone

is approved in partial fulfillment of the requirements for the degree of
Master of Science in Exercise Physiology

Examination Committee Chair
Dean of the Graduate College

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ABSTRACT

Skeletal Muscle Damage, Delayed Onset Muscle Soreness and Performance after Resistance Training with Leucine and Carbohydrate or Carbohydrate Alone

by

Matthew Steven Stock

Dr. John C. Young, Examination Committee Chair
Professor of Kinesiology
University of Nevada, Las Vegas

The purpose of this study was to evaluate the efficacy of adding leucine to pre and post-exercise carbohydrate beverages on blood markers of muscle damage, muscle soreness, and squat performance. Eighteen resistance-trained subjects performed 6 sets of squats to fatigue using 75% of the 1-RM with 3 minutes rest between sets. Subjects consumed a carbohydrate beverage (.25 g/kg) 30 minutes before and immediately after exercise with or without the addition of leucine (45 mg/kg) in randomized, double-blind fashion. Creatine kinase (CK), lactate dehydrogenase (LDH), and subjective surveys of muscle soreness, were analyzed before, 24 hrs, 48 hrs, and 72 hrs after exercise. Subjects repeated the squat protocol 72 hrs after the initial bout to test short-term recovery. The addition of leucine did not significantly decrease CK and LDH activity or soreness at 24, 48, or 72 hrs post-exercise. No differences were noted in repetitions performed between groups during the initial bout or 72 hrs post-exercise. This study suggests that the addition of leucine to carbohydrate beverages does not enhance recovery in resistance-trained subjects.
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ACKNOWLEDGMENTS

This thesis could not have been possible without the love of my parents, Steve and Diane, and sister, Stephanie. Thank you for always encouraging me to follow my dreams. This project is also dedicated to my girlfriend, Laura Leigh, for your tireless love and devotion. Thank you, Dr. Young, for your patience and guidance in acting as my committee chair and advisor. Thank you, Drs. Tandy, Golding, and Kruskall, for playing key roles in this project, as well as my professional development at UNLV. Janice Klaassen and Dr. Debra Keil also deserve special thanks for their assistance with lab space and blood analysis during this thesis, as well as my summer 07 pilot study. I am also grateful for help from UNLV friends Kevin Finnegan, Ben D’Antonio, and Missi Brown. Ultimately I believe this project, as well as everything I do in life, is a result of my faith in Jesus Christ.
CHAPTER ONE

INTRODUCTION

Leucine is an essential amino acid, and can be obtained in the diet from foods rich in protein such as chicken, fish, beef, and milk (Rose, 1957; Schaafsma, 2005). Leucine, isoleucine and valine, the branched-chain amino acids (BCAA), make up about one-third of muscle protein (Scriver, Gregory, Sovetts, & Tissenbaum, 1985). Of these amino acids, leucine has been the most thoroughly investigated because its oxidation rate is higher than that of isoleucine or valine (Bergstrom, Furst, & Hultman, 1985; Mero et al., 1997). Consumption of BCAA before or during exercise may prevent or decrease the net rate of protein breakdown (Tipton & Wolfe, 1998; Tischler, Desautels, & Goldberg, 1982; Buse & Reid, 1975, Carli et al., 1992), may improve both mental and physical performance (Blomstrand, 2006; Newsholme & Blomstrand, 1996; Blomstrand, Hassmen, Ekblom, & Newsholme, 1991), and may have a sparing effect on muscle glycogen degradation and depletion of muscle glycogen stores (Ivy, Res, Sprague, & Widzer, 2003; Blomstrand, Ek, & Newsholme, 1996; Zawadzki, Yaspelkis, & Ivy, 1992). Essential amino acids have been shown to stimulate protein synthesis after exercise (Borsheim, Tipton, Wolf, & Wolfe, 2002; Rasmussen, Tipton, Miller, Wolf, & Wolfe, 2000), and leucine plays a key role in this process independent of plasma insulin (Anthony, Anthony, & Layman, 1999; Fujita et al., 2007; Rennie, 2007). Consequently, leucine supplementation has been theorized to improve recovery and stimulate muscle
growth during resistance training. Exercise-induced muscle damage, first described by Hough in 1902, is characterized by delayed-onset muscle soreness (DOMS), Z-line streaming, general myofilament disorganization, impaired force production, and the appearance of muscle proteins in the blood (Armstrong, 1986; Clarkson & Hubal, 2002; Hought, 1902). Strategies to reduce the amount of damage a muscle incurs during exercise are poorly understood. BCAA ingestion during endurance exercise decreases the appearance of muscle enzymes in the blood, which indicates a potential reduction in muscle damage (Coombes & McNaughton, 2000). The influence of acute leucine ingestion on skeletal muscle damage and delayed-onset muscle soreness after a resistance-training session has yet to be established. In theory, if leucine ingestion reduces exercise-induced muscle damage, a decrement in exercise performance should be prevented in the days following the initial ingestion.

Statement of the Problem

1) When ingested before and after a resistance-training session, does the addition of 45 mg/kg leucine to a commercially available sports drink decrease muscle damage and subjective ratings of delayed-onset muscle soreness?

2) Does the addition of 45 mg/kg leucine to a commercially available sports drink improve exercise performance in the days following a resistance training bout above that of carbohydrate-electrolyte alone?
Null Hypothesis

1) There will be no difference between groups in blood creatine kinase values at 0, 24, 48, or 72 hours post-exercise.

2) There will be no difference between groups in blood lactate dehydrogenase values at 0, 24, 48, or 72 hours post-exercise.

3) There will be no difference between groups in subjective muscle soreness at 0, 24, 48, or 72 hours post-exercise.

4) There will be no difference between groups in squat repetitions during the initial exercise bout, as well as 72 hrs post-exercise.

Definition of Terms

1. Branched-chain amino acids (BCAA) - the combination of leucine, isoleucine and valine make up approximately 1/3 of skeletal muscle in the human body, and are the main amino acids used as fuel by exercising muscle.

2. Cortisol - a corticosteroid hormone produced by the adrenal cortex, cortisol acts as a physiological antagonist to insulin by decreasing glycogenesis and promotes breakdown of lipids, and proteins, and mobilization of extrahepatic amino acids and ketone bodies.

3. Creatine kinase (CK) - catalyses the conversion of creatine to phosphocreatine, consuming adenosine triphosphate (ATP) and generating adenosine diphosphate (ADP). After exercise creatine kinase is released by muscle into the lymphatic system where it is transported to the thoracic duct and enters the blood stream.

4. Delayed onset muscle soreness (DOMS) - the discomfort often felt after exercise that generally subsides within 2 to 3 days.

5. Eccentric - a lengthening muscle contraction in which a weight or resistance is being lowered.

6. Essential amino acid (EAA) - indispensable amino acid needed for the synthesis of body proteins which can only be obtained from the diet. They must be available simultaneously in the correct proportion for protein synthesis to take place efficiently.
7. Insulin - an anabolic hormone whose presence informs the liver and muscle cells to take in glucose and store it in the form of glycogen, and causing fat cells to take in blood lipids and turn them into triglycerides.

8. Lactate dehydrogenase (LDH) - an enzyme that catalyzes the conversion of lactate to pyruvate, LDH is commonly used to indirectly assess muscle damage after exercise.

9. Leucine - a nutritionally essential amino acid found in protein. Of the twenty amino acids, leucine plays the most significant role in the process of skeletal muscle protein synthesis.

10. Muscle damage - muscle injury in humans that frequently occurs after unaccustomed exercise, particularly if the exercise involves a large amount of eccentric contractions. These minute tears induce release of muscle proteins into the blood, inflammation, DOMS, muscle spasms, and force loss.

11. Protein degradation - the directed breakdown of proteins to amino acids by cellular enzymes called proteases.

12. Protein synthesis - the process in which individual amino acids, whether of exogenous or endogenous origin, are connected to each other in peptide linkage in a specific order dictated by the sequence of nucleotides in DNA.

13. Sarcomere - the basic unit of a muscle's cross-striated myofibril, a sarcomere is defined as the segment between two neighboring Z-lines.

**Assumptions**

1. Subjects will be sedentary on the week following 1-RM testing, and during the 72 hours following the initial exercise bout.

2. During exercise, all subjects will be self-motivated to perform to the best of their abilities.

3. Subjects will be honest during the recruitment process about their history of exercise, supplement, and anabolic steroid use.

4. Subjects are consuming a diet consisting of adequate calories and nutrients.

5. Subjects will report adverse events to lab staff.
Delimitations

The study was conducted within the following parameters:

1. Healthy, resistance-trained individuals (ages 18-50, over 1 yr full-body training ≥ 2 days/weeks) will participate in this study.

2. Subjects will be recruited from the UNLV student-body and general public by flyers posted in local gyms and coffee shops.

3. All familiarization and testing sessions will be conducted in the Exercise Physiology Laboratory in the Department of Kinesiology and the University of Nevada, Las Vegas.

4. A battery of tests will be conducted pre-supplement intervention and throughout the course of the study to determine the changes in the metabolic variables being examined.

5. Each participant will be randomly assigned to one of two supplement groups.
Friden, Sjostrom, & Ekblom (1981) provided some of the first information on muscle damage after exercise. Five healthy males ran down 10 flights of stairs 10 times with the only rest occurring when the subjects went back up to the tenth floor by elevator. In addition to muscle soreness, muscle biopsy analysis showed myofibrillar disturbances and Z-line streaming 2 and 7 days after exercise. In a later study by Friden, Sjostrom, & Ekblom (1983), muscle samples were taken 1 hour, 3 days, and 6 days after backwards cycling exercise. The authors found that 32 %, 52 %, and 12 %, respectively, of the observed fibers showed evidence of focal disturbance. Changes in ultrastructural integrity were noted as Z-line streaming, Z-lines out of register, loss in thick myofilaments, loss in mitochondria in areas that showed abnormalities, and disturbed arrangement of the A-band. According to McCully & Faulkner (1985), this initial ‘micro-damage’ is likely to be the result of high sheering forces developed during the eccentric contraction within the muscle causing damage to the smaller weaker areas of the fiber. The relatively small amount of damage is then amplified over the next few days.

Stauber, Clarkson, Fritz, & Evans (1985) studied biopsy samples from the biceps brachii taken 48 hours after subjects performed eccentric exercise to examine changes in the extracellular matrix. A decrease in resting muscle length was found immediately after
exercise that continued up to 48 hours post-exercise. It was proposed that myofiber
disruption allows intracellular proteins to escape and extracellular proteins and ions to
enter, causing swelling, whereas the disrupted extracellular matrix initiates the
inflammatory response

Muscle damage after exercise has been documented directly by myofibrillar
disruption and indirectly by the perception of soreness and a prolonged loss in strength
and range of motion (Clarkson, 1992; Newham, 1985). Repeating the same bout of
exercise (using the same muscle group) 2 weeks to several months later results in a
reduced response to the exercise such that the loss in strength and range of motion are not
as prolonged and less soreness is experienced. However, Clarkson & Tremblay (1988)
have shown that the CK response is completely negated following a second bout.
Schwane, Johnson, Vandenakker, & Armstrong (1983) were the first to complete a
systematic study of possible associations between DOMS and plasma enzyme increases
while comparing the differences displayed between level running and running down an
incline. Subjective sensations of muscular soreness and plasma activities of CK and LDH
were assessed in seven male subjects at 0, 24, 48, and 72 hours after two separate 45
minute sessions of running. Downhill running resulted in marked subjective muscle
soreness and a significant increase in plasma CK during recovery, whereas little change
was seen in the level running trial. No change was seen in LDH for either condition.

Byrnes et al. (1985) evaluated perceived muscle soreness ratings, serum creatine
kinase (CK) activity, and myoglobin levels in three groups of subjects following two 30-
minute exercise bouts of downhill running (-10 degrees slope). The two bouts were
separated by 3, 6, and 9 weeks for groups 1, 2, and 3, respectively. Criterion measures
were obtained pre- and 6, 18, and 42 hours post-exercise. On bout 1 the three groups reported maximal soreness at 42 hours post-exercise. Also, relative increases in CK for groups 1, 2, and 3 were 340, 272, and 286 %, respectively. Corresponding values for myoglobin were 432, 749, and 407 %. When the same exercise was repeated, significantly less soreness was reported and smaller increases in CK and myoglobin were found for groups 1 and 2. For example, the % CK increases on bout 2 for groups 1 and 2 were 63 and 62, respectively. Group 3 demonstrated no significant difference in soreness ratings, CK activities, or myoglobin levels between bouts 1 and 2. It was concluded that performance of a single exercise bout had a prophylactic effect on the generation of muscle soreness and serum protein responses that lasts up to 6 weeks.

Nosaka & Newton (2002) compared the magnitude of muscle damage between maximal and submaximal eccentric loading. Eight untrained male students performed 3 sets of 10 repetitions of maximal eccentric contractions with one arm and 50 % of maximal eccentric contraction with the other arm, separated by 4 weeks. In the maximal loading protocol, the elbow joint was forcibly extended from a flexed (90°) to a full-extended position in 3 seconds while producing maximal force. For submaximal loading, a dumbbell set at 50% of the maximal isometric strength at 90° of the elbow joint was lowered from the flexed to the extended position in 3 seconds. Maximal isometric force, relaxed and flexed elbow joint angle, range of motion of the elbow joint, circumference of the upper arm, muscle thickness of the elbow flexors by ultrasound images, muscle soreness, and plasma creatine kinase activity were used as markers of muscle damage. Data was collected before, immediately after, and for 5 days after submaximal and maximal eccentric loading protocols. The changes seen as a result of submaximal
eccentric loading were not significant, and faster recovery was seen. The authors concluded that maximal eccentric loads produce greater changes in markers of muscle damage than submaximal loading.

Chapman, Newton, Sacco, & Nosaka (2006) examined whether the velocity of eccentric exercise affected the magnitude of muscle damage. Twelve untrained subjects performed a series of slow velocity isokinetic eccentric elbow flexions (30°/second) of one arm and a fast velocity exercise (210/second) of the other arm, separated by 14 days. The amount of muscle actions for the slow-velocity exercise was 30 and 210 for fast velocity exercise in an attempt to standardize the total time under tension between groups. Criterion measures were range of motion, maximal voluntary torque for isometric exercise, concentric (4 velocities) and eccentric contractions (2 velocities), relaxed elbow joint angle, upper arm circumference, muscle soreness and plasma creatine kinase. Measures were taken before, immediately after, 30 minutes and 24 - 168 hours (240 hours for CK) after each eccentric exercise protocol, and changes in the measures over time were compared between velocities. Both protocols resulted in significant decrements in isometric and dynamic torque, but fast velocity exercise showed significantly greater reductions over time and a slower recovery compared to the slow velocity exercise. Significantly larger decreases in, and delayed recovery of, range of motion and relaxed elbow joint angle were evident after fast velocity exercise compared to slow velocity exercise. Fast velocity exercise had significantly larger increases in upper arm circumference and soreness, and peak plasma CK activity was 4.5 times greater compared to slow velocity exercise. This study suggests that fast velocity eccentric exercise causes greater muscle damage than slow velocity exercise.
The ability to perform at a high level in the days after strenuous exercise is of utmost importance to athletes and coaches. In a study by Twist & Eston (2005), 10 male subjects performed 10 x 6 second cycle ergometer sprints with 24 seconds of rest between trials against a load corresponding to 0.10 kp/kg and 10 x 10 meter sprints from a standing start, each with 12 seconds of walking between trials. All variables were measured immediately before and at 30 minutes, 24, 48 and 72 hours following a plyometric exercise protocol comprising of 10 sets of 10 maximal counter movement jumps. Significant changes over time were seen for perceived soreness, plasma creatine kinase activity, peak power output, sprint time and rate of fatigue. Soreness was significantly higher than baseline values at all time intervals. CK was significantly elevated at 24 and 48 hours compared to baseline. The results provide evidence that, following a plyometric, muscle-damaging exercise protocol, the ability of the muscle to generate power is reduced for at least 3 days.

*Protein and Amino Acid Supplementation as a means of Decreasing Post-exercise Muscle Damage and Improving Performance*

A recent study investigated the effects of 6-weeks of leucine supplementation in male and female competitive outrigger canoeists (Crowe, Weatherson, & Bowden, 2006). It was hypothesized that 45 mg/kg/d leucine would improve anthropometric characteristics, endurance and power performance and reduce RPE in comparison to placebo, and that these changes would be accomplished via a decreased plasma ratio of free tryptophan to BCAA. Ten female and three male (1 withdrew after an unrelated injury occurred) outrigger canoeists were treated with leucine or placebo in randomized,
double-blind fashion. All subjects were asked to maintain their normal training and diet, in addition to keeping a daily food intake journal throughout the course of the study.

Post-leucine testing results showed a significantly improved performance in upper-body 10s peak power and row to exhaustion, as well as a significant decrease in RPE compared to placebo. There was not a significant difference between groups in anthropometric measurements and the ratio of free-tryptophan to BCAA, making the mechanism behind the improved performance seen in the leucine group poorly understood. Although not studied, the authors speculated that the improved performance was caused by reduced training-induced muscle damage and enhanced recovery after intense training, giving the subjects in the leucine group the ability to perform at a higher level than the control group for the 6 week study period.

Baty et al. (2007) investigated the effect of a 6.2% carbohydrate-1.5% protein solution on resistance-training performance, hormonal response, and muscle damage. The two specific goals of the study were to examine whether supplementing with a carbohydrate-protein beverage before and during a resistance training bout would increase the number of repetitions performed, and to assess blood creatine kinase and myoglobin in an attempt to help understand the mechanisms for any potential improved performance. Thirty-four males that had not engaged in resistance training and not taken any dietary supplements over the preceding 6 months participated in the study. In double-blind, posttest-only control group design, subjects consumed 355 ml of either the CHO-protein treatment drink or placebo 30 minutes prior to exercise, 177 ml immediately prior to exercise, 177 ml halfway through the bout, and 355 ml immediately after the exercise bout. Subjects completed 3 sets of 8 repetitions of 7 exercises incorporating all the large
muscle groups of the limbs and trunk using their 8-RM. The ability to lift more than 8 repetitions on the third set of any exercise was defined as an improvement in performance. No significant difference in exercise performance was seen between groups. Cortisol and creatine kinase were significantly increased at 24 hours post-exercise in the placebo group. Myoglobin levels were significantly elevated in the placebo group at 6 hours post-exercise, but had returned to normal by 24 hours post-exercise. These results suggest that ingestion of a carbohydrate-protein beverage may have little impact on acute resistance training performance, but appears to improve muscle damage, as indicated by creatine kinase and myoglobin.

Saunders, Kane, & Todd (2004) compared the effects of a carbohydrate-protein solution to a carbohydrate-only solution on cycling time to fatigue and muscle damage. Fifteen trained male cyclists (V0₂ peak of ≥ 40 ml/kg/min) volunteered for the study. Each subject performed two prolonged bouts of cycle ergometry to fatigue with 12 to 15 hours of rest period between rides. The first exercise bout was performed at 75 % V0₂ peak, while the second was performed at 85 % V0₂ peak. Subjects ingested 1.8 ml/kg of preconstituted treatment fluid (CHO-protein or CHO-only) every 15 minutes of exercise and 10 ml/kg of treatment fluid within 30 minutes after the exercise bouts. The CHO-protein beverage utilized a 4:1 ratio and the beverages were matched for CHO content, resulting in 20 % lower total caloric content for the CHO-only administration. The subjects returned after a 7 to 14-day washout period and exercised again using the opposite treatment condition. Subjects rode 29 % longer when consuming the CHO-protein beverage than the CHO-only beverage in the first trial. Subjects performed 40 % longer as a result of CHO-protein consumption than the CHO consumption in the second
trial. Peak post-exercise plasma CK levels were 83% lower after the CHO-protein trial than the CHO-only trial.

Romano-Ely, Todd, Saunders, & Laurent (2006) used a very similar design as Saunders et al. (2004), but used isocaloric treatments. Fourteen male cyclists were studied to compare the effect of a protein-carbohydrate-antioxidant beverage (CHOPA) to an isocaloric carbohydrate-only beverage on time-to-fatigue and post-exercise muscle damage as indicated by CK and LDH. Subjects performed two prolonged bouts of cycle ergometry to fatigue 24 hours apart. The first exercise bout was performed at 70% VO2 peak and the second was performed at 80% VO2 peak. Each subject consumed 2 ml/kg of either CHOPA or CHO every 15 minutes during exercise and immediately after. The subjects returned after a 7- to 14-day washout period and exercised again using the opposite treatment condition. There was no difference in time-to-fatigue at 70% VO2 peak, 80% VO2 peak, or total performance time between CHOPA and CHO treatments, despite the higher carbohydrate content of the CHO-only treatment. Post-exercise CK and LDH values were significantly lower in the CHOPA trials than the CHO trial, suggesting that the addition of a small amount of protein may aid trained cyclists with post-exercise muscle damage.

Flakoll, Judy, Flinn, Carr, & Flinn (2004) studied the effectiveness of post-exercise protein supplementation on recruit health, muscle soreness, and function during the stress of Marine 54-day basic training. Three hundred eighty-seven male recruits from six Marine platoons were randomly assigned to three treatments within each platoon in double-blind fashion. Nutrients supplemented immediately post-exercise during the 54-day basic training were either placebo (0 g carbohydrate, 0 g protein, 0 g fat), control (8,
Subjective rating of muscle soreness immediately post-exercise was reduced by protein supplementation vs. placebo and control groups on both days 34 and 54. Compared with placebo and control groups, the protein group had an average of 33% fewer total medical visits, 28% fewer visits due to bacterial/viral infections, 37% fewer visits due to muscle/joint problems, and 83% fewer visits due to heat exhaustion, suggesting that individuals undergoing extreme physical exertion may benefit from a post-exercise protein supplement.

Nosaka, Sacco, & Mawatari (2006) investigated the effect of a supplement containing 9 essential and 3 non-essential amino acids on muscle damage and soreness using two separate ingestion patterns (Experiment 1 and Experiment 2). Thirty-eight male students who were non-athletes, free from any musculo-skeletal disorders, and had not been involved in any regular resistance training for at least 1 year before this study were recruited. Each subject went through two exercise bouts using opposite arms separated both 3-4 weeks. The exercise bout consisted of 30 minutes of continuous flexion and extension of the elbow flexors at 9% of the subjects' maximal isometric strength determined at an elbow joint of 90 degrees. In Experiment 1, fourteen subjects ingested a supplement containing 3.6g of amino acids (60% essential amino acids, of which were 60% BCAAs), 10 vitamins, 0.5g CHO and a small amount of fat, or a placebo, before, and immediately after exercise. In Experiment 2, twenty-four subjects took the supplements before, immediately after exercise, and at night (after dinner) for the exercise day and after breakfast and dinner for 3-days following exercise and after breakfast on day 4 after exercise. Subjects from Experiment 2 ingested a total of 36g amino acids over the 5-day period. The authors hypothesized that the ingestion pattern in Experiment 1 would not be
sufficient to enhance recovery after the exercise bout. Maximal voluntary contraction, range of motion, upper arm circumference, creatine kinase, aldolase activities, myoglobin, subjective muscle soreness, and concentration of amino acids in the blood were measured immediately before, immediately after, and 1, 6, 24, 48, 72, and 96 hours following exercise. As hypothesized, there were no statistically significant changes in any of the criterion measures in Experiment 1. However, muscle soreness, plasma creatine kinase, aldolase, myoglobin, and muscle soreness were significantly lower for the amino acid condition in Experiment 2. These results suggest that supplementing with 3.6g amino acids before and after exercise will have little effect in untrained subjects, but continuing such a supplement protocol for several days may assist in the recovery of skeletal muscle after a resistance training session.

Kraemer et al. (2006) tested the effects of amino acid supplementation during short-term resistance training overreaching in double-blind, placebo-controlled, randomized fashion. Overreaching occurs as a result of intensified training and is often considered a normal outcome for elite athletes due to the relatively short time needed for recovery. Seventeen resistance-trained men were randomly assigned to either an amino acid or a placebo group and underwent four weeks of resistance training over-reaching in an attempt to minimize recovery between workouts. Subjects assigned to the amino acid group ingested .4g/kg/d of essential and conditionally essential amino acids. All subjects were matched for diet and were approximately at the typical American diet of 55 % carbohydrates, 30 % fat, and 15 % protein for basic composition with no differences between groups. Resistance training overreaching was performed on 4 consecutive days using a total-body program for 4 weeks. The first 2 weeks consisted of moderate-intensity
work, while the last 2 weeks consisted of a high-intensity, lower-volume resistance exercise protocol. Each subject provided a venous blood sample and had 1RM squat and bench press assessed before and at the completion of each training week. A major finding of the study was that significant elevations in creatine kinase were observed in the placebo group after the first week of training. This elevation in creatine kinase was correlated highly to reductions in 1-RM squat, indicating a potential relationship between muscle damage and performance. These changes were not observed in the amino acid group. Interestingly, the elevations in creatine kinase seen quickly returned close to normal after the first week of training and remained so throughout the course of the study, suggesting that these resistance-trained subjects quickly adapted to the exercise stimulus. The practical application of these results suggests that athletes that will be engaged in several weeks of intense training (2-a-day football camp, etc.) may benefit from amino acid supplementation, particularly early-on in the training phase.

Coombes & McNaughton (2000) tested the hypothesis that BCAA supplementation would reduce serum creatine kinase and lactate dehydrogenase after prolonged exercise. Sixteen recreationally active male subjects were assigned to one of two groups: the supplemental group (12g/d BCAA for 14 days) or the control group. Unique to other similar studies, one of the aims of this experiment was to ensure that all subjects were consuming a recommended BCAA intake of .64g/kg/d. This value was obtained by using the highest recommendation of protein intake of 1.6g/kg/d (Lemon et al, 1991) and the suggestion from Adibi (1976) that BCAA should make up 40% of protein intake. Subjects kept dietary intake logs throughout the course of the study and study investigators called the subjects each night. If caloric or protein intake were too
low, the subjects were encouraged to drink milk. Baseline creatine kinase and lactate dehydrogenase were taken prior to the commencement of the study. On day seven each subject cycled on an ergometer for 120 minutes at approximately 70% VO2max. Blood samples were taken before and immediately after exercise, as well as 1, 2, 3, 4, 24, 48, 72, 120 and 168 hours post-exercise. Data analysis demonstrated that all subjects consumed the recommended daily intake of BCAA (.64 g/kg) in their diet. The main findings of this study were that the serum activities of the intramuscular enzymes were reduced following BCAA supplementation. The serum CK activity increased to maximum values at 24 hours post-exercise by approximately 275% in the control group but only 175% in the BCAA group. LDH activity increased to maximum values at 4 hours post-exercise by approximately 55% in the control group but only by 40% in the supplemented subjects.

Ohtani, Maruyama, Suzuki, Sugita, & Kobayashi (2001) focused on the physiological and biochemical changes before, during and after long-term amino acid supplementation in collegiate endurance athletes. The aim of the study was to determine an appropriate dosage of amino acids for enhancing performance and recovery. Thirteen male athletes at the University of Tokyo were studied over a 6 month period. The subjects were advised to follow their normal diet throughout the course of the study and the level of training remained constant. A 12 amino acid mixture featuring 9 essential amino acids, glutamine and arginine was used in the study. The daily doses of amino acid mixture were 2.2 g/day, 4.4 g/day and 6.6 g/day. Each subject was tested at all 3 dosages by partitioning the experimental period into 3 separate 2 month blocks. Each 2 month block featured a month of supplementation and a washout block. Blood samples were
taken at the beginning and end of every supplement period. Subjects gave their opinion of their physical condition using a 5-grade scale (5=excellent, 4=very good, 3=good, 2=bad, 1=very bad) by a .5 increment before and after each period. The activity of creatine kinase was unaltered as a result of 2.2 and 4.4g amino acids/day for one month. 6.6 g/day significantly decreased creatine kinase levels.

Shimomura et al. (2006) evaluated whether pre-exercise BCAA supplements might attenuate muscle soreness and muscle fatigue induced by exercise. Sixteen female and fourteen male untrained subjects were studied in double-blind, cross-over fashion on two occasions separated by 12 weeks. Each subject ingested a 5g BCAA mixture (Isoleucine: Leuice: Valine = 1:2.3:1.2), or placebo 15 minutes prior to exercise. The exercise protocol consisted of 7 sets of 20 repetitions of squats (140 total repetitions) with 3 minutes of rest between sets. Subjective rating of muscle soreness was evaluated before, after and for the 4 days after exercise with the use of a visual-analogue scale consisting of a 10-cm line with “no pain” printed at one end and “extremely sore” at the other. Muscle fatigue was assessed at the same time in the same manner. Muscle soreness in the female placebo trial was significantly elevated in the second and third days following exercise. Men exhibited a lesser degree of muscle soreness for the BCAA trial than the placebo at all time points after exercise, but the differences were not statistically significant. It was speculated that this was due to the higher relative BCAA intake in the female subjects compared to the males (92 ± 2 mg/kg for females and 77 ± 3 mg/kg for males). Muscle fatigue was also lower for BCAA supplementation than placebo in both females and males.
Sugita, Ohtani, Ishii, Maruyama, & Kobayashi (2003) investigated the effect of an amino acid mixture on strength performance after eccentric exercise. Twenty-two male students participated in the study. The experimental design was a double-blind crossover test, with an amino acid mixture and a placebo given orally and twice daily, as the treatment. Two separate 10-day experiments were conducted 2 months apart. The eccentric exercise protocol consisted of 3 sets of 8 maximal eccentric exertions using an isokinetic dynamometer in which the arm was moved from 30 degrees to 105 degrees at a speed of 10 rpm. Maximal isometric, concentric, and eccentric strength were measured before, immediately after, and 1, 2, 3, 5, 6, and 10 days later. Amino acid supplementation significantly improved isometric contraction muscle strength of the elbow extensors on days 2, 3, and 6 after the exercise bout. No other statistically significant differences were seen between amino acid supplementation and placebo in any of the other parameters examined.

**Nutritional Interventions to Promote Post-Exercise Muscle Protein Synthesis**

Feeding induces an anabolic response in skeletal muscle of growing postabsorptive animals (Yoshizawa, 1998), and amino acids play a key role in mediating this response. It is clear that the provision of amino acids coupled with a resistance training bout result in an increase in muscle protein synthesis above that of muscle protein breakdown, resulting in a positive net muscle protein balance. It is also understood that non-essential amino acids do not increase muscle protein balance (Borsheim et al., 2002; Tipton, Ferrando, Phillips, Doyle, & Wolfe, 1999) and recent investigations have discovered the importance of the branched-chain amino acid leucine.

Muscle protein accretion occurs in the recovery phase after exercise rather than during the actual exercise period (Carraro, Stuart, Hartl, Rosenblatt, & Wolfe, 1990; Rennie et al., 1980). Because studies had shown an elevated rate of muscle protein synthesis after exercise but had not examined muscle protein breakdown, Biolo, Maggi, Williams, Tipton, & Wolfe (1995) suggested that an acute resistance training bout had to also increase muscle protein breakdown, or much greater increases in muscle growth would occur as a result of resistance training. Five untrained male volunteers were studied on two separate occasions. On the first occasion subjects were studied at rest. For the second occasion each subject was studied after an intense lower-body resistance training bout. Stable isotopic tracers of amino acids in combination with the catheterization of femoral vessels and the biopsy of the vastus lateralis were used. Muscle protein synthesis was elevated after exercise, as was muscle protein breakdown, but to different extents. Consequently, muscle protein balance improved after exercise but did not shift into a positive state, suggesting that other factors, such as feeding, are necessary to promote muscle anabolism.

Phillips, Tipton, Aarsland, Wolf, & Wolfe (1997) were the first to examine the time course of the responses of muscle protein net balance after an isolated bout of resistance exercise. Eight volunteers that had not engaged in any forms of resistance training for \( \geq 5 \) months were studied on four occasions: at rest, 3 hours post-exercise, 24 hours post-exercise, and 48 hours post-exercise. For the three non-rest experiments, each
subject performed 8 sets of 8 concentric or eccentric repetitions at 80% of their predetermined 1-RM on the leg extension machine. Exercise resulted in significant increases in muscle protein synthesis at all 3 time points after exercise. Muscle protein breakdown was increased at 3 and 24 hours post-exercise, but returned to baseline 48 hours post-exercise. All time points revealed a negative net balance, which was expected because the subjects were told to maintain a meat-free diet and were fasting during all data collection.

Hyperaminoacidemia resulting from intravenous infusion of amino acids results in muscle protein synthesis at rest (Biolo, Tipton, Klein, & Wolfe, 1997). Due to the limited practical application of such practice, Tipton et al. (1999) tested the hypothesis that an orally administered solution of amino acids would increase protein synthesis after resistance training. It was also hypothesized that because the availability of non-essential amino acids is not greatly diminished after exercise, non-essential amino acids would not be necessary to include in a supplement designed to increase muscle protein synthesis after exercise. Six subjects with no resistance training experience volunteered for the study. On three separate occasions, each subject went through an intense lower-body resistance training bout. After each session, each subject consumed a placebo supplement, 40g mixed amino acids (21.4g essential amino acids, 18.6 g non-essential amino acids), or 40g essential amino acids in small increments over a 3-hour period. Muscle biopsies were collected throughout the study. It was determined that orally administered amino acids did in fact increase muscle protein synthesis above breakdown, and that non-essential amino acids do not play a role in protein synthesis. Interestingly, the change seen in the essential amino acid trial was not much greater than that seen in the mixed amino acid trial, suggesting that there is a maximum rate at which protein
synthesis can be achieved after resistance exercise. The authors suggested that despite the higher arterial amino acids concentrations seen in the essential amino acid trial, the translational machinery of the cell is not capable of increasing protein synthesis above a maximum level.

Rasmussen et al. (2000) tested the interactive effect between amino acid availability, insulin, and resistance exercise. It was hypothesized that ingestion of an essential amino acid-carbohydrate drink would have an optimal effect on muscle protein metabolism. The authors compared the response to the treatment at two different time intervals. Six recreationally-active volunteers (3 men, 3 women) went through a lower-body resistance-exercise protocol and consumed 6g essential amino acids, 35g CHO drink at 1 or 3 hours after exercise. Each subject went through the protocol twice and served as his or her own control. Results showed that while muscle protein breakdown was unchanged, muscle protein synthesis increased, thereby significantly increasing net balance above pre-exercise levels at both 1 and 3 hour time points. No significant difference was found between the two, suggesting that the precise timing of ingestion in relation to the exercise stimulus was not of consequence. The authors noted that although transient, the combination of essential amino acids, CHO and resistance exercise used in this study was the highest protein synthetic rate reported in any circumstance.

Previous work has shown that amino acids play a critical role in muscle protein metabolism after exercise, making them a key component to a dietary supplement designed to promote anabolism. However, the importance of caloric intake coupled with free amino acids was less clear. Prior work by Biolo, Williams, Fleming, & Wolfe, (1999) had determined that after resistance training, local hyperinsulinemia caused no
further stimulation of muscle protein synthesis. Miller, Tipton, Chinkes, Wolf, & Wolfe (2003) hypothesized that this was due to a relative insufficient supply of amino acids, and assessed the individual and interactive effects of amino acids and/or CHO on muscle protein synthesis after exercise. A second purpose of the study was to determine if the response to a second bolus of amino acids and/or CHO was affected by the prior ingestion of the same dose 1 hour earlier. This was examined in response to a study by Bohe, Low, Wolfe, & Rennie (2001) which had recently found that during a 6-hr intravenous infusion of amino acids, protein synthesis was only elevated for the first 2 hours. Ten volunteers participated in the study. On three separate occasions, subjects went through a resistance training session. The training session consisted of the leg press (10 sets of 10 repetitions) and knee extension (8 sets of 8 repetitions) exercise performed at 75 % of the 1-RM. At 1 and 2 hr post exercise, each subject consumed one of three beverages: CHO, Mixed Amino Acids, or CHO/mixed amino acids. The amount of carbohydrate and amino acids was adjusted according to body weight, based on 35g CHO and 6g essential amino acids per 70kg subject. The amino acid mixture was approximately 50 % essential amino acids (2.8 g essential amino acids for every 6g amino acids). When 35g CHO was combined with 6g essential amino acids, the effect of net muscle protein balance was roughly equivalent to the sum of their independent responses. These results suggest that after exercise, insulin increases the potential for accelerated muscle protein synthesis, but this can only be reflected if amino acid availability is increased simultaneously. Furthermore, the response to ingestion of the second dose of each drink at 2 hours after exercise was essentially the same as the response to the first dose.
Borsheim, Tipton, Wolf, & Wolfe (2002) set out to determine the independent effect of ingestion of a bolus of 6g essential amino acids at both 1 and 2 hour intervals after resistance training. The results of this study were then compared to the response with previous research (Miller et al., 2003) which used a balanced mixture of amino acids (3g essential amino acids and 3g non-essential amino acids). Six recreationally active subjects went through an exercise protocol consisting of 10 sets of 10 repetitions of leg presses and 8 sets of 8 repetitions of leg extensions using 80% of their tested 1-RM with 2 minutes of rest between sets. At 1 and 2 hours post-exercise, the subjects were given an oral supplement consisting of .087g essential amino acids/kg/body weight. The nutritional supplement was designed to increase intramuscular essential amino acid availability in proportion to muscle protein. The anabolic response to this particular supplement was about twice as much as the mixed amino acid supplement used by Miller et al. (2003), suggesting that there is a dose-response to the amount of essential amino acids ingested post-exercise. Ingestion of the two post exercise drinks at 1 hr and 2 hr intervals had no effect on one another. Isoleucine and leucine blood concentrations increased more so than other amino acids and therefore the goal of causing proportional increases in all essential amino acids was not achieved. Glycine and alanine concentrations did decrease, but due to the fact that that an essential amino acid dose-response was seen, these amino acids were not rate-limiting.

The response to ingestion of 100g CHO on net muscle protein balance has been examined (Borsheim et al., 2004). It had been previously shown that the response of muscle protein synthesis to 6g essential amino acids did not appear to differ with the addition of 35g CHO (Rasmussen et al., 2000). Sixteen recreationally active subjects
volunteered for the study. Subjects performed 10 sets of 8 reps of leg extensions at 80% of their 1-RM and ingested either 100g CHO in the form of maltodextrin or placebo 1 hour after exercise. The principal finding was that 100g CHO after resistance exercise improved net protein balance. No change was seen in muscle protein synthesis, making the improvement in net balance solely a result of decreased muscle protein breakdown. The authors cautioned that the improvement was of questionable physiological significance because the net balance did not reach positive values. Furthermore, the improvement was minor when compared with past studies using essential amino acids (Borsheim et al., 2002; Tipton, Ferrando, Phillips, Doyle, & Wolfe, 1999).

While the majority of research conducted in this area has examined the muscle protein response after exercise, Tipton et al. (2001) compared pre and post resistance training supplementation. It was hypothesized that hyperaminoacidemia from ingestion during the exercise bout, as opposed to after, might counter the net loss of muscle protein, thereby creating a more anabolic state. Six recreationally active subjects volunteered for the study and each participated in two trials in random order. Muscle biopsies were taken before and at the end of an exercise protocol consisting of 10 sets of 8 repetitions of leg presses and 8 sets of 8 repetitions of leg extensions at 80% of the 1-RM. Between the initial muscle biopsy and initiation of exercise, subjects consumed a 500 ml bolus of either 6g essential amino acids and 35g sucrose or placebo. The subjects then consumed the opposite drink immediately after exercise. It was determined that the total response to the consumption of the drink was greater when essential amino acids and carbohydrate were consumed immediately before exercise as opposed to immediately after. This difference was speculated to be due to increased amino acid availability. Thus the authors
concluded that providing amino acids at a time when blood flow is elevated, such as during exercise, maximizes delivery to the muscle.

Tipton et al. (2004) were the first to examine the response of net muscle protein balance to ingestion of whole proteins after exercise and did so by comparing the response of two different whole proteins, casein and whey. Casein is emptied from the stomach more slowly than whey and is therefore considered a “slow” protein. Amino acids from casein appear in the blood more slowly, and peak at a lesser magnitude, but the response lasts longer than with whey proteins (Boirie et al., 1997). Young, healthy subjects were split into three groups: placebo (N=7), 20g casein (N=7) and whey (N=9). Each group went through an exhaustive lower-body exercise protocol lasting approximately 25 minutes and ingested their respective drink 60 minutes after exercise. Muscle biopsies from the vastus lateralis were taken to measure intramuscular amino acid concentrations immediately before exercise, 55 minutes post exercise (immediately before consumption of the drink), 120, and 300 minutes after exercise. Leucine and phenylalanine concentrations were measured in femoral arteriovenous samples to determine balance across the leg. Peak leucine net balance over time was greater for whey than for casein. There was no significant difference in net phenylalanine balance in response to the two drinks. The pattern of amino acid appearance in the blood was markedly different for casein than for whey proteins.

Tipton et al. (2007) researched the response of muscle protein to whole proteins before or after resistance training, and compared it to that of free amino acids from a past study (Tipton et al, 1999). Unlike the response to essential amino acids in the previous study, the anabolic response to whey protein ingestion was similar whether ingested
before or following exercise. It seems that the timing of whey ingestion in relation to the exercise is not as important as essential amino acids, and when comparing the two it appears that amino acid delivery during exercise is greater when essential amino acids are ingested than when whey protein is ingested immediately before exercise.

**Summary**

Unaccustomed or strenuous exercise has been shown to induce damage to skeletal muscle fibers, particularly when the activity has a high degree of eccentric loading. The extent of damage that occurs is primarily dependent on an individual’s training experience, as well as the intensity, volume, and type of training session. The symptoms of exercise-induced muscle damage are common and include delayed-onset muscle soreness, stiffness, swelling, and decreased force output. Exercise-induced muscle damage is also characterized by increased intramuscular enzyme appearance in the blood plasma or serum. Creatine kinase, lactate dehydrogenase, troponin I, and myoglobin have been commonly used to assess muscle damage in the hours, days, and weeks after exercise. Attempts to reduce or eliminate the amount of damage a muscle incurs during exercise have been relatively unsuccessful. When compared to carbohydrate or placebo, ingestion of protein and amino acids before and after resistance training improves net muscle protein balance. The amino acid leucine augments this anabolic response in skeletal muscle, suggesting a potential role for leucine in reducing exercise-induced muscle damage.
CHAPTER THREE

METHODOLOGY

Resistance trained subjects performed six sets of squats to muscular failure with three minutes of rest between sets using 75% of their pre-determined 1-RM in an attempt to induce muscle damage. Each subject ingested .25g CHO/kg of a commercially available carbohydrate-electrolyte sports drink thirty minutes prior to and immediately after the training session. The independent variable was the addition of 22.5 mg/kg leucine powder to both the pre- and post-exercise test beverages. Dependent variables included blood levels of creatine kinase and lactate dehydrogenase and muscle soreness at 24, 48, and 72 hours post-exercise, in addition to the number of repetitions performed 72 hours after the initial exercise bout.

Subjects

Eighteen healthy, resistance-trained individuals (ages 18-50, over 1 yr full-body training ≥ 2 days/weeks) participated in this study. Subjects were not allowed to participate in this study if they had any had a history of heart disease, hypertension, diabetes, thyroid disease, hypoglycemia, or musculoskeletal disease or injury. Subjects were also not allowed to participate if they had consumed ergogenic levels of nutritional supplements that may affect muscle mass [e.g., creatine, beta alanine, hydroxyl-beta-methylbutarate (HMB)] or anabolic/catabolic hormones (androstenedione, DHEA, etc.)
within three months prior to the study. The study was approved by the University Institutional Review Board for Human Subjects, and all subjects completed a health history questionnaire and signed a written informed consent document before testing.

Study Site

All familiarization assessments, subjective surveys, exercise sessions and blood draws were conducted in the Exercise Physiology Laboratory at the University of Nevada, Las Vegas.

Familiarization Session & Strength Testing

Subjects eligible to participate in the study were familiarized to the study protocol via verbal and written explanation outlining the study design. During this session, subjects signed an Informed Consent Statement and completed personal and medical questionnaires. To ensure subject compliance, the familiarization session also included a complete description the exercise protocol and verbal assurance from potential subjects that he/she is experienced in squatting. Following the familiarization session and at least 7 days before the research protocol, one-repetition maximum (1-RM) squat testing was performed. The 1-RM was defined as the maximal amount of weight that could be safely lifted one time without the use of a spotter. Seventy-five percent of the 1-RM was then used as the constant load for the research protocol. Intensity was based on a percentage of the 1-RM as opposed to multiple-repetition testing in an effort to avoid the repeated bout effect displayed in prior studies (McHugh, 2003; Paddon-Jones, Muthalib, & Jenkins, 2000).
Randomization and Supplementation Protocol

Subjects were randomly assigned into two groups ingesting, in double-blind manner, 25 mg/kg of a carbohydrate-electrolyte sports drink with or without the addition of 45 mg/kg leucine powder. Subjects ingested their prospective beverage in the presence of a study investigator thirty minutes prior to and immediately after a lower-body resistance training bout.

Analytical Methods

Blood Creatine Kinase and Lactate Dehydrogenase

Exercise-induced muscle damage studies have shown elevations in blood enzymes at up to 96 hours post-exercise (Faulkner & Brookes, 1997). Based on pilot work using 6 resistance-trained subjects, we determined that 6 sets of squats to fatigue with 3 minutes of rest between sets using 75% of the pre-determined 1-RM was a sufficient protocol to elevate blood creatine kinase and lactate dehydrogenase.

Five ml of blood was drawn from the antecubital vein immediately before supplement ingestion, as well as 24, 48, and 72 hours post-exercise. Samples were immediately centrifuged and frozen at -80 degrees Celsius until analysis. At the conclusion of the data collection all samples were thawed at room temperature and processed on a Cirrus automated chemistry analyzer (Stanbio, Boerne, TX).
Subjective Muscle Soreness

A 7-point Likert scale of muscle soreness was given to each subject before, as well as 24, 48, and 72 hours after the first exercise bout (Vickers, 1999). The scale ranged from 1 (no soreness present) to 7 (a severe pain that limits ability to move). The survey was used to discern psychological aspects of the study. Post-exercise values were compared with baseline values and between treatment groups.

Exercise Performance

On the initial day of testing, subjects performed 6 sets of barbell squats to muscular failure using a 75% of their 1-RM with 3 minutes of rest between sets. In an attempt to identify any difference in recovery between treatment groups, subjects returned to the Exercise Physiology Laboratory 72 hours after the initial bout and repeated the exercise bout again. Repetitions were counted during both exercise trials and compared between groups. Both groups were supervised by study coordinators during all training sessions.

Diet Analysis

Subjects were given food logs to self-report energy intake over the 72 hour period after the initial squat test (Day 1). Subjects were asked to follow their normal diet over the study period and to consume whole foods and avoid protein powders, drinks, and bars. Food logs were then analyzed for average protein content between groups with the use of The Food Processor SQL (ESHA Research, Salem, OR).
Statistical Analysis

The study was a double-blind, posttest-only control group design, to test for main and interaction effects (treatment x time). Diet records were analyzed by using an independent-group t-test. The blood analysis portion of the experimental design was a between-within mixed model design (2 x 4), in which the between-subjects factor was the drink (Leucine-CHO or CHO) and the within-subjects factors was timing (blood draws 1–4). Both initial testing and 72 hr post-exercise squat performance was analyzed by using an independent group t-test. Muscle soreness was analyzed with a mixed-model ANOVA. The alpha level was set at p = 0.05. All data are presented as means ± SE.
CHAPTER FOUR

RESULTS

Subject Data

While 20 subjects completed the trial, data from 2 subjects was removed due to high levels of blood creatine kinase and lactate dehydrogenase at the initiation of the study, suggesting potential noncompliance with the study protocol. All other participants attended and completed all training sessions. Therefore, data from 18 participants (n = 9 LCHO; n = 9 CHO-only) has been presented in this study.

Eighteen resistance trained subjects with an average (±SD) age, height, and body mass of 22.6 (3.02) yrs, 69.0 (3.60) in, and 79.77 (15.48) kg, respectively, participated in the study. Subjects had 4.47 (2.39) yrs of resistance training experience and had an average 1-RM squat of 134.94 (41.22) kg. No significant differences exist between groups for resistance training experience or 1-RM squat (p> .05).

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<td>Height (in)</td>
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<td>RT experience (yrs)</td>
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<td>1-RM squat (kg)</td>
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Serum Creatine Kinase

Serum creatine kinase (U/I) was less in the LCHO group than the CHO group at 24 (569.2 for LCHO; 729.0 for CHO), 48 (528.1 for LCHO; 689.9 for CHO), and 72 hrs (467.7 for LCHO; 599.8) post-exercise, but the differences were not significantly significant (F=.215, p = .8293). Serum creatine kinase was elevated as a result of the exercise protocol and both groups followed the same trend across all time points. This elevation was significant at 24 hrs in both groups (p = .001), but declined at 48 (p = .068) and 72 hrs (p = .082) to levels that were not significantly increased over baseline.
Serum lactate dehydrogenase (IU/L) was not significantly different between groups at any of the post-exercise time points ($F = 1.529, p = .234$). Little relative change was observed 24 hrs post-exercise in both groups ($p = 1.00$), suggesting that lactate dehydrogenase does not follow the same time course as creatine kinase after resistance exercise. Whereas LCHO increased modestly from baseline at all time points, a robust but non-significant ($t = -.99, p = .17$) increase was observed from 48 to 72 hrs in the CHO group.
Exercise Performance

Exercise performance, as defined as the maximum number of repetitions performed, was not significantly different between groups at initial testing (p=.43), as well as 72 hrs post-exercise (p=.40) suggesting the addition of leucine to carbohydrate had no effect on exercise performance. Participants in the LCHO group performed an average (+ SD) 55.4 (18.06) repetitions over the 6 sets of squats during initial testing, while subjects in the CHO group performed 54.0 (17.40). After 72 hrs of rest, the LCHO group performed 46.1 (20.62) repetitions, and the CHO performed 48.6 (21.04) repetitions.
Delayed Onset Muscle Soreness

Subjective ratings of delayed onset muscle soreness was not effected as a result of the addition of leucine to carbohydrate (F=.705, p =.554). All subjects experienced muscle soreness post-exercise as a result of the exercise regimen. Whereas muscle soreness peaked and did not change from 24 to 48 hrs post-exercise in the LCHO group, soreness in the CHO group peaked at 48 hrs post-exercise.

Diet Analysis

The diets of all 18 subjects were analyzed for protein content. The average self-reported protein intake in the LCHO group was 1.55 g/kg/d, whereas the average self-reported protein intake in the CHO group was 1.64 g/kg/d. Independent group t-tests revealed no significant difference between groups (p = .35).
Self-reported protein intake (3 day average)

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g/kgbw
CHAPTER FIVE

DISCUSSION

The major finding of this study is that ingestion of a leucine-carbohydrate beverage before and after resistance exercise did not significantly decrease blood creatine kinase and lactate dehydrogenase activity compared to carbohydrate alone. Additionally, the ingestion of leucine and carbohydrate did not significantly improve exercise performance, as determined by the number of repetitions performed during 6 sets of squats during initial testing and 72 hrs later. Delayed onset muscle soreness was not significant between groups at any of the time points tested.

Studies examining the effectiveness of nutritional interventions on blood markers of muscle damage and soreness have shown conflicting results, and Mero (1999) proposed that caution must be paid when interpreting studies using leucine as a whole protein or branched-chain amino acid mixture. Creatine kinase has been commonly used as an indirect marker of muscle damage due to it’s magnitude of increase relative to other enzymes, as well as it’s relatively low cost of analysis.

Ingestion of a carbohydrate-protein beverage before, during, and after exercise has been shown to decrease creatine kinase and myoglobin and favorably alter cortisol and insulin after resistance training compared to placebo (Baty et al., 2007). Similar carbohydrate-protein studies using trained cyclists have found significantly lower creatine kinase compared to an isocarbohydrate treatment (Saunders et al., 2004) and
isocaloric carbohydrate treatment (Romano-Ely et al., 2006). This study only examined the effectiveness of a pre- and post-exercise nutritional supplement, and found no effect of leucine ingestion. Other studies (Kraemer, Volek, Bush, Putukian, & Sebastianelli, 1998, Nosaka et al., 2006) have found a decrease in creatine kinase after several days of amino acid supplementation. Coombes et al. (2000) found a decrease in blood creatine kinase and lactate dehydrogenase after cycling with 14 days of branched-chain amino acid supplementation and a high protein diet. Green and colleagues (2008) found no improvement in creatine kinase with carbohydrate or carbohydrate-protein feeding above placebo after downhill running in female subjects. Millard-Stafford et al. (2005) also failed to find a significant difference in creatine kinase between carbohydrate-protein and carbohydrate following a 21-km run plus a treadmill run to fatigue at 90% VO2max. Whereas our study compared leucine-carbohydrate to carbohydrate alone, Greer, Woodard, White, Arguello, & Haymes (2007) found that carbohydrate ingestion attenuated creatine kinase activities at 24 and 48 h postexercise as compared with a placebo beverage.

Few studies have examined the effects of leucine alone on exercise performance. Wingate test performance and knee extensor strength in wrestlers (Mourier et al., 1997), muscle size and strength in older men (Godard., Williamson, & Trappe, 2002) and countermovement jump performance in power athletes (Mero et al. 1997) were unaffected by varying periods of dietary leucine or branched-chain amino acid supplementation when compared to placebo conditions. Pitkänen and colleagues (2003) found no effect on acute performance with leucine supplementation in male power athletes. More recently, a 6 week trial of leucine supplementation in competitive
outrigger canoeists found significant improvements in endurance performance and upper body power, but no improvement in heart rate or anthropometric variables (Crowe et al., 2006). The authors suggested that the improved performance was related to either an increased rate of protein synthesis or a reduction in exercise-induced muscle damage over the course of 6 weeks.

Ingestion of carbohydrate during prolonged exercise is well accepted as a means of improving endurance performance (Wright, Sherman, & Dernbach, 1991; Costill, 1991; Costill et al., 1981). However, little attention has been given to carbohydrate or amino acid ingestion and acute resistance training performance (Conley & Stone, 1996). In agreement with the results of the present study, others (Baty et al., 2007; Kraemer et al., 2006) have not seen an improvement in acute resistance training performance with protein-carbohydrate supplementation. Three studies have shown improved performance with carbohydrate supplementation and resistance training (Haff et al., 2001; Haff et al., 1999; Lambert et al., 1991), while two have found no improvement (Conley et al., 1995; Vincent, Clarkson, Freedson, & Decheke, 1993). In a recent review, Haff, Lehmkuhl, McCoy, & Stone (2003) suggested that for carbohydrates to improve exercise performance the exercise bout had to be longer than 50 minutes in duration and needed to focus on one major group of muscles. Previous work has determined that a typical resistance training bout does not reduce blood glucose (Keul, Haralambie, Bruder, & Gottstein, 1978), and given that all subjects in the present study consumed carbohydrates before exercise, the decline in exercise performance seen in both groups was likely a result of exercise-induced muscle damage and not carbohydrate availability. The limited amount of data on macronutrient ingestion and acute resistance training performance
provides an unclear relationship. Haff et al. (2003) speculated that pre, during, and post resistance-training carbohydrates may provide an ergogenic benefit only if the exercise bout is high in volume and intensity, but concluded that much more research is necessary.

The final aim of the present study was to evaluate the effectiveness of adding leucine to carbohydrate before and after resistance training on subjective assessment of delayed onset muscle soreness with the use of a 7-point Likert scale. It was determined that subjects in the leucine-carbohydrate group did not experience significantly less delayed onset muscle soreness at 24, 48, and 72 hrs post-exercise. Though delayed onset muscle soreness is typically present 24-72 hrs after exercise (Clarkson & Hubal, 2002), the duration and magnitude of pain can vary from subject to subject. In a review of measurement tools used in muscle damage research, Warren, Lowe, & Armstrong (1999) concluded that soreness was the most commonly used marker of injury, and that 63% of these studies assessed soreness subjectively either through the use of a visual analogue scale or a 7-point Likert scale. Though supplement trials have failed to eliminate delayed onset muscle soreness or directly influence muscle recovery, some studies have shown moderate improvement. Nosaka et al. (2006) determined that a supplement containing 9 essential and 3 non-essential amino acids decreased delayed onset muscle soreness, but only if the supplement period lasted several days after the initial exercise bout. A study in 387 United States Marine recruits found that muscle soreness immediately post-exercise was reduced by protein supplementation vs. placebo and control groups (Flakoll et al., 2004). Greer and colleagues (2007) found that branched chain amino acids significantly attenuated delayed onset muscle soreness 24 hrs after exercise compared to carbohydrate and placebo, but not 4 or 48 hrs after exercise. The results from the present study suggest
that delayed onset muscle soreness is not improved or eliminated by adding leucine to a pre- and post-exercise carbohydrate beverage.

In conclusion, this study does not support the initial hypothesis that the addition of leucine to a carbohydrate beverage enhances recovery after resistance training. The limited amount of leucine studies on exercise outcomes suggests that this topic deserves further research. Furthermore, reviewers should use caution when comparing studies that use different subject pools (trained vs. untrained), methods of inducing muscle damage, and protein-amino acid mixtures.
APPENDIX

Blood Creatine Kinase Data

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<td>3103</td>
<td>4059</td>
<td>1449</td>
<td>2505</td>
<td>3116</td>
</tr>
<tr>
<td><strong>Day 3 Protein (g)</strong></td>
<td>162</td>
<td>186</td>
<td>45</td>
<td>137</td>
<td>132</td>
</tr>
<tr>
<td><strong>Day 3 Protein per kg body-weight</strong></td>
<td>1.92</td>
<td>2.31</td>
<td>0.66</td>
<td>1.94</td>
<td>1.52</td>
</tr>
<tr>
<td>3 Day Average Protein per kg body-weight</td>
<td>Subject 12</td>
<td>Subject 13</td>
<td>Subject 14</td>
<td>Subject 15</td>
<td>Subject 16</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td></td>
<td>1.60</td>
<td>1.97</td>
<td>0.86</td>
<td>1.94</td>
<td>1.64</td>
</tr>
<tr>
<td>Body-weight (kg)</td>
<td>88.64</td>
<td>71.14</td>
<td>71.4</td>
<td>81.81</td>
<td>89.09</td>
</tr>
<tr>
<td>Day 1 Calories (kcal)</td>
<td>3196</td>
<td>2195</td>
<td>2044</td>
<td>3283</td>
<td>3629</td>
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<tr>
<td>Day 1 Protein (g)</td>
<td>149</td>
<td>147</td>
<td>114</td>
<td>170</td>
<td>240</td>
</tr>
<tr>
<td>Day 1 Protein per kg body-weight</td>
<td>1.68</td>
<td>2.07</td>
<td>1.60</td>
<td>2.08</td>
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<tr>
<td>Day 2 Calories (kcal)</td>
<td>4596</td>
<td>1811</td>
<td>4718</td>
<td>3808</td>
<td>4720</td>
</tr>
<tr>
<td>Day 2 Protein (g)</td>
<td>229</td>
<td>91</td>
<td>188</td>
<td>198</td>
<td>240</td>
</tr>
<tr>
<td>Day 2 Protein per kg body-weight</td>
<td>2.58</td>
<td>1.28</td>
<td>2.63</td>
<td>2.42</td>
<td>2.69</td>
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<tr>
<td>Day 3 Calories (kcal)</td>
<td>2654</td>
<td>1071</td>
<td>2202</td>
<td>1240</td>
<td>4720</td>
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<tr>
<td>Day 3 Protein (g)</td>
<td>100</td>
<td>106</td>
<td>89</td>
<td>74</td>
<td>240</td>
</tr>
<tr>
<td>Day 3 Protein per kg body-weight</td>
<td>1.13</td>
<td>1.49</td>
<td>1.25</td>
<td>0.90</td>
<td>2.69</td>
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<tr>
<td>3 Day Average Protein per kg body-weight</td>
<td>1.80</td>
<td>1.61</td>
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<td>2.69</td>
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<tr>
<td></td>
<td>Subject 17</td>
<td>Subject 19</td>
<td>Subject 20</td>
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</tr>
<tr>
<td>Body-weight (kg)</td>
<td>125</td>
<td>82.27</td>
<td>92.27</td>
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<td>Day 1 Calories (kcal)</td>
<td>2000</td>
<td>4817</td>
<td>1197</td>
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<td></td>
</tr>
<tr>
<td>Day 1 Protein (g)</td>
<td>166</td>
<td>183</td>
<td>48</td>
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<td></td>
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<tr>
<td>Day 1 Protein per kg body-weight</td>
<td>1.33</td>
<td>2.22</td>
<td>0.52</td>
<td></td>
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</tr>
<tr>
<td>Day 2 Calories (kcal)</td>
<td>2400</td>
<td>2391</td>
<td></td>
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<tr>
<td>Day 2 Protein (g)</td>
<td>110</td>
<td>111</td>
<td>60</td>
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<td></td>
</tr>
<tr>
<td>Day 2 Protein per kg body-weight</td>
<td>0.88</td>
<td>1.35</td>
<td>0.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3 Calories (kcal)</td>
<td>2650</td>
<td>2000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3 Protein (g)</td>
<td>155</td>
<td>104</td>
<td>41</td>
<td></td>
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<td>Day 3 Protein per kg body-weight</td>
<td>1.24</td>
<td>1.26</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Day Average Protein per kg body-weight</td>
<td>1.15</td>
<td>1.61</td>
<td>0.54</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Subjective Assessment of Muscle Soreness

Please check next to the box that best describes your answer to each question. You may only choose one answer to each question.

### 24 hrs post exercise
What was your level of muscle soreness over the past 24 hours?
1. A complete absence of muscle soreness
2. A light pain felt only when touched/a vague ache
3. A moderate pain felt only when touched/a slight persistent pain
4. A light pain when walking up or down stairs
5. A light pain when walking on a flat surface/painful
6. A moderate pain, stiffness, or weakness when walking/very painful
7. A severe pain that limits my ability to move

### 48 hrs post exercise
What was your level of muscle soreness over the past 24 hours?
1. A complete absence of muscle soreness
2. A light pain felt only when touched/a vague ache
3. A moderate pain felt only when touched/a slight persistent pain
4. A light pain when walking up or down stairs
5. A light pain when walking on a flat surface/painful
6. A moderate pain, stiffness, or weakness when walking/very painful
7. A severe pain that limits my ability to move

### 72 hrs post exercise
What was your level of muscle soreness over the past 24 hours?
1. A complete absence of muscle soreness
2. A light pain felt only when touched/a vague ache
3. A moderate pain felt only when touched/a slight persistent pain
4. A light pain when walking up or down stairs
5. A light pain when walking on a flat surface/painful
6. A moderate pain, stiffness, or weakness when walking/very painful
7. A severe pain that limits my ability to move
Biomedical IRB – Full Board Review
Approval Notice

NOTICE TO ALL RESEARCHERS:
Please be aware that a protocol violation (e.g., failure to submit a modification for any change) of an IRB approved protocol may result in mandatory remedial education, additional audits, re-consenting subjects, researcher probation suspension of any research protocol at issue, suspension of additional existing research protocols, invalidation of all research conducted under the research protocol at issue, and further appropriate consequences as determined by the IRB and the Institutional Officer.

DATE: February 13, 2008
TO: Dr. John Young, Kinesiology
FROM: Office for the Protection of Research Subjects
RE: Notification of IRB Action
Protocol Title: Skeletal Muscle Damage, Delayed Onset Muscle Soreness and Performance After Resistance Training with Leucine and Carbohydrate or Carbohydrate Alone
Protocol #: 0712-2567

This memorandum is notification that the project referenced above has been reviewed by the UNLV Biomedical Institutional Review Board (IRB) as indicated in Federal regulatory statutes 45CFR46. The protocol has been reviewed and approved.

The protocol is approved for a period of one year from the date of IRB approval. The expiration date of this protocol is January 21, 2009. Work on the project may begin as soon as you receive written notification from the Office for the Protection of Research Subjects (OPRS).

PLEASE NOTE:
Attached to this approval notice is the official Informed Consent/Assent (IC/IA) Form for this study. The IC/IA contains an official approval stamp. Only copies of this official IC/IA form may be used when obtaining consent. Please keep the original for your records.

Should there be any change to the protocol, it will be necessary to submit a Modification Form through OPRS. No changes may be made to the existing protocol until modifications have been approved by the IRB.
Should the use of human subjects described in this protocol continue beyond January 21, 2009, it would be necessary to submit a **Continuing Review Request Form 60 days** before the expiration date.

If you have questions or require any assistance, please contact the Office for the Protection of Research Subjects at OPRSHumanSubjects@unlv.edu or call 895-2794.
TITLE OF STUDY: Skeletal Muscle Damage, Delayed Onset Muscle Soreness and Performance after Resistance Training with Leucine and Carbohydrate or Carbohydrate Alone

INVESTIGATOR(S): John C. Young (Principal Investigator), Matthew S. Stock (Student Investigator, Lawrence Golding, Richard Tandy, Laura Kruskall

CONTACT PHONE NUMBER: John C. Young, 895-4626

Purpose of the Study
You are invited to participate in a research study. Past research indicates that ingestion of amino acids after strength training plays a pivotal role in the metabolic processes of increasing muscle mass and improving recovery. The purpose of this thesis project is to examine the efficacy of adding a commercially-available amino acid supplement to a pre-and post-exercise sports drink for the purposes of decreasing exercise-induced muscle damage and soreness, and improving exercise performance in the days following the initial bout of exercise.

Participants
You are being asked to participate in the study because you are a healthy individual between the ages of 18 and 50 with at least twelve months of resistance training experience. You may not participate in this study if you have any history of heart disease, hypertension, diabetes, thyroid disease, hypoglycemia, or musculoskeletal disease or injury. Potential subjects may not participate if they have consumed ergogenic levels of nutritional supplements that may affect muscle mass [e.g., creatine, beta alanine, hydroxyl-beta-methylbutarate (HMB)] or anabolic/catabolic hormones (androstenedione, DHEA, etc.) within three months prior to the study. Individuals that have used anabolic steroids at any point in their life will be excluded from the study. Pregnant females and females that may become pregnant will be excluded from the study as well. The results from this thesis project would have great health and human performance implications for athletes of all ages, parents, coaches, and even the elderly.

Procedures
If you volunteer to participate in this study, you will be asked to do the following: Prior to initiation of the study, the researchers will determine your lower-body strength. One week later you will participate in a maximal-effort lower-body exercise protocol
designed to induce muscle damage and soreness. Before and after the training session you will ingest a commercially available carbohydrate-electrolyte sports drink with or without the addition of an amino acid. The amount of amino acid will be 45 milligrams per kilogram of bodyweight (22.5 mg before and 22.5 mg after exercise), which is approximately 3.5 grams for a 170-lb individual. Neither you nor the researcher will know the composition of your test beverage. You will be required to keep a dietary-intake journal for the 3 days following the exercise bout. Immediately before, and 24, 48, and 72 hrs after exercise, you will answer questions in a subjective survey related to your feelings of muscle soreness and discomfort. If you consent to participate, a venipuncture (blood sample collection) will be done to draw a maximum of 10ml of blood from your forearm vein using a 21 gauge needle (standard size) immediately before, as well as 24, 48, and 72 hrs after exercise. The blood will be used to test levels of creatine kinase and lactate dehydrogenase, which are indirect markers of skeletal muscle damage after exercise. At 72 hrs post-exercise, you will go through the initial exercise bout again. All exercise bouts require maximal effort. You must refrain from physical exercise throughout the course of the study. The total amount of time needed from you will be no greater than five hours.

**Expected Subject Schedule**

Day 1- 1-RM Squat testing
Day 2- Subject remains sedentary
Day 3- Subject remains sedentary
Day 4- Subject remains sedentary
Day 5- Subject remains sedentary
Day 6- Subject remains sedentary
Day 7- Subject remains sedentary
Day 8- Baseline Blood Draw (creatine kinase and lactate dehydrogenase) and subjective survey 35 minutes before exercise.
Subject ingests test drink 30 minutes before exercise.
Exercise: 8 sets of barbell squats to muscular failure using pre-determined 10-RM with 3 min rest between sets. Exercise must be performed with maximal effort.
Subject ingests test drink immediately after exercise.
Day 9 (24 hrs post)- Subject remains sedentary; blood draw (creatine kinase and lactate dehydrogenase), subjective survey
Day 10 (48 hrs post)- Subject remains sedentary; blood draw (creatine kinase and lactate dehydrogenase), subjective survey
Day 11 (72 hrs post)- Blood draw (creatine kinase and lactate dehydrogenase), subjective survey before exercise.
Exercise: 8 sets of barbell squats to muscular failure using pre-determined 10-RM with 3 min rest between sets. Exercise must be performed with maximal effort.

**Benefits of Participation**

There will be no direct benefit to you as a participant in this study. The researchers hope to learn more about the efficacy of leucine supplementation in resistance-trained individuals and a general overview of the study results will be shared.
Risks of Participation
There are risks involved in all research studies. Upon completion of exercise, you may experience slight pain in your legs, dizziness, fatigue, shortness of breath, and temporary muscle soreness. The risks of having blood drawn include minor bleeding, bruising, and fainting. All of these rarely occur and do not cause any significant problems. The amino acid used in this study is an essential amino acid that is found in protein-rich foods such as chicken and milk. It must be consumed in the diet for maintenance of muscle mass and strength. The specific amino acid used in this study has been examined in over 50 clinical trials across the world, and at this point it appears that there are no side effects, risks, or adverse events that have occurred due to its ingestion.

Due to the nature and intensity of exercise used in this study, there is minor risk of musculoskeletal injury involved with participation. This risk will be greatly minimized by the fact that all subjects must be trained and experienced in lower-body resistance training. Prior to subject participation you will be instructed on proper technique. Risk will also be minimized through the use of supervision and highly-skilled spotters that are familiar with resistance training. Additionally, each subject will participate in a standardized warm-up and cool-down before and after all exercise sessions. If at any time during the study the researchers feel that a subject is at risk of injury because of poor exercise technique or inability to follow instructions, the subject will be removed from the study.

Cost/Compensation
There will not be financial cost to you to participate in this study. The study will take approximately 5 total hours of your time on 5 separate days. You will not be compensated for your time. The University of Nevada, Las Vegas may not provide compensation or free medical care for an unanticipated injury sustained as a result of participating in this research study.

Contact Information
If you have any questions or concerns about the study, you may contact Matt Stock or John Young at 954-801-7308 and 702-895-4626. For questions regarding the rights of research subjects, any complaints or comments regarding the manner in which the study is being conducted you may contact the UNLV Office for the Protection of Research Subjects at 702-895-2794.

Voluntary Participation
Your participation in this study is voluntary. You may refuse to participate in this study or in any part of this study. You may withdraw at any time without prejudice to your relations with the university. You are encouraged to ask questions about this study at the beginning or any time during the research study.

Confidentiality
All information gathered in this study will be kept completely confidential. No reference will be made in written or oral materials that could link you to this study. All records
will be stored in a locked facility at UNLV's Exercise Physiology Laboratory (MPE326) for 3 years after completion of the study. After the storage time the information gathered will be shredded and disposed of.

Your blood sample will be assigned a code number and the key to the code will be maintained by the principal investigator. Records of this study will be stored in a locked file cabinet at UNLV and kept for a minimum of three years after the completion of the study. The University of Nevada, Las Vegas Biomedical Sciences IRB will have access to these records. Your identity will not be revealed to any unauthorized persons and will be protected to the extent allowed by law. You will not be personally identified in any reports or publications that may result from this study.

**Participant Consent:**
I have read the above information and agree to participate in this study. I am at least 18 years of age. A copy of this form has been given to me.

_________________________________________  _________________________________
Signature of Participant               Date

_________________________________________
Participant Name (Please Print)

*Participant Note: Please do not sign this document if the Approval Stamp is missing or is expired.*
REFERENCES


Nosaka, K., & Newton, M. (2002). Difference in the magnitude of muscle damage between maximal and submaximal eccentric loading. *Journal of Strength and


Matthew Steven Stock

Home Address:
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Davie, FL 33328

Degrees:
Bachelor of Science degree in Exercise Science and Health Promotion
Florida Atlantic University, Davie, FL

Thesis Title:
Skeletal Muscle Damage, Delayed Onset Muscle Soreness and Performance after
Resistance Training with Leucine and Carbohydrate or Carbohydrate Alone

Thesis Examination Committee:
Chairperson, Dr. John C. Young, Ph. D.
Committee Member, Dr. Lawerence Golding, Ph. D.
Committee Member, Dr. Dick Tandy, Ph. D.
Graduate Faculty Representative, Dr. Laura Kruskall, Ph. D., RD