

EFFECT OF LEUCINE SUPPLEMENTATION ON INDICES OF
MUSCLE DAMAGE AND RECOVERY FOLLOWING
ECCENTRIC-BASED RESISTANCE EXERCISE

A Thesis
by
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ABSTRACT

EFFECT OF LEUCINE SUPPLEMENTATION ON INDICES OF MUSCLE DAMAGE AND RECOVERY FOLLOWING ECCENTRIC-BASED RESISTANCE EXERCISE (May 2010)

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In vitro, the amino acid leucine has been able to reduce proteolysis and be a potent stimulus for protein synthesis. The purpose of this study was to determine the effect of leucine supplementation on indices of muscle damage and muscular function following eccentric-based resistance exercise. Twenty-seven untrained individuals (Height: 178.62 ± 5.54 cm; Mass: 77.71 ± 13.46 kg) were randomly divided into 3 groups; leucine (L), placebo (P) and control (C). The L and P groups performed 100 depth jumps from 60 cm and 6 sets of 10 repetitions of eccentric-only leg presses. Either leucine (250 mg/kg bm) or placebo was ingested at 3 time points during exercise and each recovery day following exercise. Each group's level of muscle damage was determined via serum levels of creatine kinase (CK) and myoglobin (MYO) at pre-exercise (PRE) and 24, 48, 72 and 96 hours post-exercise. Muscle function was determined by peak force (PF) during an isometric squat (ISO) and jump height (JH) and peak concentric force (PCF)

during both a static jump (SJ) and countermovement jump (CMJ) at PRE and 24h, 48h, 72h and 96h post-exercise. CK was significantly elevated from PRE (354.8 ± 317.5 U/L) for the L group at 24h (713.4 ± 473.0 U/L) and significantly elevated from PRE (185.9 ± 117.7 U/L) for the P group at 72h (501.7 ± 434.3 U/L) and 96h (455.6 ± 326.0 U/L). MYO was significantly elevated from PRE (24.03 ± 15.4 ng/ml) for the P group at 24h (66.3 ± 59.6 ng/ml) and 96h (52.9 ± 41.3 ng/ml). PF for the ISO significantly decreased across all time points for P group and 24h, 48h, and 72h for the L group. SJ JH significantly decreased from PRE (0.42 ± 0.05 m) for the P group at 24h (0.38 ± 0.04 m), 48h (0.38 ± 0.06 m) and 72h (0.39 ± 0.04 m). CMJ JH significantly decreased from PRE (0.46 ± 0.03 m) for the L group at 48h (0.42 ± 0.04 m), 72h (0.43 ± 0.03 m) and 96h (0.39 ± 0.14 m) and also from PRE (0.48 ± 0.05 m) for the P group at 24h (0.44 ± 0.08 m), 48h (0.43 ± 0.07 m) and 72h (0.43 ± 0.06 m). SJ PCF significantly increased from PRE (1652.1 ± 226.0 N) for the L group at 48h (1728.9 ± 264.8 N), 72h (1725.8 ± 279.5 N) and 96h (1730.0 ± 270.0 N) and was significantly higher compared to the P group at 24h and 48h. CMJ PCF significantly increased from PRE (1673.6 ± 308.7 N) for the P group at 48h (1760.6 ± 399.5 N). Leucine supplementation does not appear to be a viable choice for reducing immediate muscle damage following eccentric exercise; however leucine may facilitate the recovery process. Leucine may need further examination, as ingestion of leucine did not result in a between group difference compared to P but did minimize the change in muscle damage markers compared to PRE. Supplementing with leucine by itself may not completely reduce the levels of muscle damage that follow high-intensity resistance training, but may help maintain force generating capabilities.

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TABLE OF CONTENTS

	Page
ABSTRACT.....	iv
ACKNOWLEDGEMENTS.....	vi
LIST OF TABLES.....	ix
LIST OF FIGURES.....	x
INTRODUCTION.....	1
REVIEW OF LITERATURE.....	3
Exercise-Induced Muscle Damage.....	3
Molecular Mechanisms and Biochemical Pathways Associated with Muscle Damage and Recovery.....	8
Amino Acid Supplementation and Exercise Protocols.....	14
METHODOLOGY.....	20
Subjects.....	20
Study Design.....	20
Muscle Function Assessment.....	21
1RM Strength Testing.....	23
Resistance Exercise Protocol.....	23
Supplement Protocol.....	24
Blood Collection.....	24
Biochemical Analysis.....	25
Muscle Function Analysis.....	26
Perceived Muscle Soreness.....	27
Dietary Analysis.....	27
Statistical Analyses.....	27
RESULTS.....	29
Biochemical.....	29
Muscle Function.....	29
Perceived Muscle Soreness.....	32
Dietary Analysis.....	32

DISCUSSION.....	33
REFERENCES	41
TABLES	59
FIGURES.....	62
APPENDIX A – Institutional Review Board Documentation.....	74
VITA.....	85

LIST OF TABLES

Table 1: Anthropometric and strength values for each group.....	59
Table 2: Jump Performance and Muscle Function for DJ20	60
Table 3: Jump Performance and Muscle Function for DJ40	61
Table 4: Jump Performance and Muscle Function for DJ60	62
Table 5: Subject Dietary Intake of kCal, Carbohydrates, Protein and Fat.....	63

LIST OF FIGURES

Figure 1: Perceived Muscle Soreness Scale.....	64
Figure 2: Serum Creatine Kinase Activity.....	65
Figure 3: Serum Lactate Dehydrogenase Activity.....	66
Figure 4: Serum Myoglobin Concentrations.....	67
Figure 5: Peak Force During Isometric Squat.....	68
Figure 6: Static Jump Performance.....	69
Figure 7: Countermovement Jump Performance	70
Figure 8: Peak Concentric Force During Static Jump	71
Figure 9: Peak Concentric Force During Countermovement Jump.....	72
Figure 10: Perceived Muscle Soreness	73

INTRODUCTION

Resistance exercise induces various physiological events to occur within the body, with many of these changes occurring within skeletal muscle. Repeatedly exposing the musculature to mechanical overload stimulates the tissue to adapt to better accommodate further overload. Resistance exercise typically consists of performing exercises that contain both an eccentric and concentric phase. The eccentric phase has been shown to induce the greatest level of damage within the muscle tissue (Gibala, MacDougall, Tarnopolsky, Stauber, & Elorriaga, 1995). The breakdown of the tissue stimulates reparative mechanisms to become activated and begin the process of tissue remodelling (Cramer et al., 2004). This breakdown of tissue can be grouped into either primary damage resulting from mechanical stress, or secondary damage from proteolytic pathways and inflammation (Armstrong, Warren, & Warren, 1991). While initial damage caused by mechanical loading may be unavoidable, the resulting cellular processes that exacerbate the secondary damage may be able to be attenuated. One way that this may be accomplished is through supplementation with nutrients that have been shown to influence these cellular processes (Cockburn, Hayes, French, Stevenson, & St Clair Gibson, 2008).

Due to the body's requirement for amino acids to produce various proteins, including those needed for tissue repair, increasing the abundance of amino acids within the body would appear to be a logical choice to facilitate skeletal tissue recovery. While

amino acids are required to produce functional proteins, they can also influence recovery through the mediation of a number of cellular processes associated with regulating skeletal muscle metabolism. While the role of amino acids on cellular signalling continues to be a highly investigated area, it does appear that certain amino acids, particularly leucine, have a greater role in these processes compared to others (Vary, Jefferson, & Kimball, 1999). Leucine is an essential amino acid and continues to be one of the most investigated amino acids due to its role in skeletal muscle metabolism, both as an anabolic agent and an anti-catabolic agent (Anthony, Anthony, Kimball, Vary, & Jefferson, 2000; Combaret et al., 2005; La Bounty, Campbell, Oetken, & Willoughby, 2008; Sugawara, Ito, Nishizawa, & Nagasawa, 2007; Ventrucci, Mello, & Gomes-Marcondes, 2004). Resistance exercise is a potent stimulus for skeletal muscle metabolism and remodelling, which suggests that leucine supplementation during resistance exercise might be of benefit. Therefore, the purpose of this investigation was to determine the effect of short-term leucine supplementation on indices of muscle damage, muscle function and perceived muscle soreness following an acute bout of eccentric exercise.

REVIEW OF LITERATURE

Exercise-Induced Muscle Damage

While a multitude of literature exists on exercise-induced muscle damage, the exact mechanisms that contribute to the damage remain poorly understood. Exercise-induced muscle damage can occur during two separate phases, often classified as the initial, or primary phase, and the secondary phase (Armstrong, et al., 1991). Primary damage occurs during the exercise bout (McHugh, 2003) and is usually categorized as either mechanical or metabolic (Armstrong, et al., 1991; Kuipers, 1994). Mechanical damage is a result of the external forces overcoming that which cannot be maintained by the contractile proteins, and thereby causing damage within the sarcomeres (Armstrong, et al., 1991). Metabolic damage is the result of free radical production during energy metabolism and causes oxidative stress to occur within the muscle (Close, Ashton, McArdle, & Maclaren, 2005). Metabolic damage is thought to only occur during exercise of long duration (Howatson & van Someren, 2008), with damage occurring by metabolic deficiencies within the working muscle (Tee, Bosch, & Lambert, 2007). Therefore, the focus will remain on muscle damage caused by mechanical factors as these mechanisms are primarily responsible for the muscle damage that occurs during bouts of high-intensity resistance exercise.

Primary Damage

Mechanical damage has been shown to occur as a result of either resistance exercise bouts that primarily involve eccentric contractions (Dolezal, Potteiger, Jacobsen, & Benedict, 2000; Gibala, et al., 1995), or novel or unaccustomed exercise (Ebbeling & Clarkson, 1989). Eccentric exercise induces muscle damage via disruption in the sarcomeres within the myofibrils and damage to entities involved in excitation-contraction coupling (Proske & Morgan, 2001). Faulkner, Brooks and Opitck (1993) suggested that damage to the sarcomeres may be a result of actin and myosin being stretched beyond overlap thereby causing damage to those sarcomeres. The current contraction state of the muscle may determine what microstructures get damaged. If forced lengthening of the muscle occurs when actin and myosin are in a bound state, damage will occur in those contractile structures as seen by increases in plasma concentrations of troponin I (Sorichter et al., 1997) and myosin heavy chain (Sorichter et al., 2001). However, during eccentric muscle contractions, actin and myosin are not always in a bound state due to overstretched sarcomeres, with the location of the overstretched sarcomeres occurring at random (Morgan, 1990). This overstretching may lead to disruption (Talbot & Morgan, 1996); with the tension of the overstretched sarcomeres being transferred to the cytoskeletal proteins titin, desmin and α -actinin (Howatson & van Someren, 2008). Therefore, these cytoskeletal proteins are additional structures that can become damaged independent from the contractile proteins. Titin is a very large-weight molecular protein that connects myosin filaments to the Z-line. Desmin is a protein that is mostly located in the Z-disks and connects adjacent Z-disks (Morgan & Allen, 1999). Desmin is sensitive to eccentric contractions with damage

occurring extremely early in exercise (Lieber, Thornell, & Friden, 1996). Alpha-actinin is a major structural protein that anchors actin-containing thin filaments and maintains spatial arrangement between myofilaments (Blanchard, Ohanian, & Critchley, 1989).

Muscle damage is thought to occur to either structural or contractile proteins; however there are other structures within the sarcomere that could sustain damage. These structures include those involved in excitation-contraction coupling such as the transverse tubules, sarcoplasmic reticulum and sarcolemma. Limited literature exists on primary damage to structures involved in excitation-contraction coupling. Results from animal models suggest that decreased contractile forces following eccentric exercise may be a result of failure to fully activate the contractile apparatus, rather than actual damage to contractile elements (Ingalls, Warren, Williams, Ward, & Armstrong, 1998; Warren et al., 1993). Therefore, this decreased contractility may be independent of damage to contractile proteins.

Secondary Damage

The main causes of secondary muscle damage are thought to be associated with the loss of intracellular Ca^{2+} homeostasis and the initiation of mechanisms associated with an overload of Ca^{2+} (Armstrong, et al., 1991) combined with inflammatory response (McHugh, 2003). An increase of Ca^{2+} within the cytosol may come from two different sources, the first being extracellular (Armstrong, et al., 1991), and the second coming from changes in the sarcoplasmic reticulum after lengthening contractions (Nielsen, Madsen, Jorgensen, & Sahlin, 2005). This large influx of intracellular Ca^{2+} initiates the mechanisms that further damage the muscle cell via alterations in the cytoskeleton, sarcoplasmic reticulum, mitochondria, and myofilaments (Gissel & Clausen, 2001).

These mechanisms include Ca^{2+} -dependent proteolytic and phospholipolytic pathways, which lead to the degradation of structural and contractile myofibrillar proteins as well the cellular membrane (Kuipers, 1994).

Indices of Skeletal Muscle Damage

Creatine kinase (CK) and lactate dehydrogenase (LDH) are metabolic enzymes located within the sarcolemma and their activity in serum has been used extensively as an indirect method of determining skeletal muscle damage (Cooke, Rybalka, Williams, Cribb, & Hayes, 2009; Coombes & McNaughton, 2000; Greer, Woodard, White, Arguello, & Haymes, 2007). CK is a dimeric globular protein which buffers ATP and ADP concentrations by catalyzing the exchange of phosphate bonds between phosphocreatine and ADP produced during muscular contraction (Brancaccio, Maffulli, & Limongelli, 2007). Basal serum levels of CK in healthy males are between 51 IU/L and 313 IU/L (Strømme, Rustad, Steensland, Theodorsen, & Urdal, 2004), however following strenuous exercise serum levels can increase dramatically as a result of muscle damage (Brancaccio, et al., 2007). Similarly, LDH is an enzyme which catalyzes the conversion of pyruvate to lactate during anaerobic glycolysis (Voet, Voet, & Pratt, 2006). Basal serum levels of LDH in healthy males are between 103 IU/L and 204 IU/L (Strømme, et al., 2004). Myoglobin (MYO) is an oxygen transport molecule located within the sarcolemma that has also been used to assess muscle damage (Childs, Jacobs, Kaminski, Halliwell, & Leeuwenburgh, 2001; Kraemer et al., 2009). Basal levels of MYO in serum range from 10 ng/mL to 68 ng/mL (Rosano & Kenny, 1977).

Alteration in Muscle Function

As mentioned previously, exercise-induced muscle damage may affect muscular function through damage to structural proteins and structures involved in excitation-contraction coupling. The combined effect results in a reduction in the ability of the muscle to contract with maximal force (Pearce, Sacco, Byrnes, Thickbroom, & Mastaglia, 1998), which is observed in all three types of muscle contraction, eccentric, concentric, and isometric (Turner, Tucker, Rogasch, & Semmler, 2008). The length of time that force production is reduced depends on a multitude of factors, including individual differences, the muscle that is damaged, and the intensity of the exercise (Byrne, Twist, & Eston, 2004). Byrne and Eston (2002b) reported a 35% decrease in knee extensor isometric strength immediately following a resistance exercise protocol consisting of 10 sets of 10 repetitions in the barbell squat with emphasis placed on the eccentric phase of the exercise. Following the initial decrement, isometric strength steadily increased in a linear fashion during the recovery days, yet still remained significantly lower on day 7 in comparison to baseline values (Byrne & Eston, 2002b). Eccentric exercise has also been shown to affect dynamic muscle contractions, such as those used during a vertical jump (Byrne & Eston, 2002a; Harrison & Gaffney, 2004). Harrison and Gaffney (2004) found that eccentric exercise significantly reduced take-off velocity and subsequent performance for static jumps, countermovement jumps, and depth jumps. Similarly, Byrne and Eston (2002a) showed a significant decrease in jumping performance in the 72 hours following a resistance exercise protocol consisting of 10 sets of 10 barbell squats. The type of vertical jump may determine the rate of recovery as static jumps which contain no eccentric phase are affected by eccentric exercise to a greater extent than

countermovement jumps or drop jumps (Byrne & Eston, 2002a; Harrison & Gaffney, 2004). Exercise-induced muscle damage also reduces peak force output during vertical jumping (Garcia-Lopez et al., 2006).

Additional investigations have utilized protocols involving exercises that emphasize the stretch-shorten cycle (SSC) to induce muscle damage (Horita, Komi, Nicol, & Kyrolainen, 1999; Kyrolainen, Takala, & Komi, 1998; Strojnik & Komi, 1998). The SSC is when a preactivated muscle is first stretched and then followed by a rapid shortening action (Nicol, Avela, & Komi, 2006). Following a SSC damage protocol, there was a marked difference in the recovery pattern for the static jump and drop jumps (Horita, Komi, Hamalainen, & Avela, 2003). Static jump performance decreased rapidly following the damage protocol and then recovered quickly, while the drop jump performance only decreased 48 and 96 hours following the exercise (Horita, et al., 2003). This is contrary to what was observed in eccentric-based muscle damage protocols, which indicated that the type of damage protocol might dictate the recovery pattern in different jumping conditions. The reduction in force-generating capability following exercise-induced muscle damage may impair the ability to perform subsequent activities requiring large amounts of force to be generated.

Molecular Mechanisms and Biochemical Pathways Associated with Muscle Damage and Recovery

Multiple molecular mechanisms contribute to cellular protein breakdown and protein synthesis. Similarly, modification of various molecules relies on the integration of multiple biochemical pathways in order to produce a specific compound. The pathways which are thought to be influenced by leucine and its metabolites include the

ubiquitin-proteasome pathway, the mevalonate pathway, and the mammalian target of rapamycin pathway (Wilson, Wilson, & Manninen, 2008).

Ubiquitin-Proteasome Pathway

The ubiquitin-proteasome pathway is one of the main mechanisms responsible for cellular protein degradation (Attaix et al., 2005). Murton, Constantin, and Greenhaff (2008) reviewed the biochemical processes associated with the ubiquitin-proteasome system (UPS). The UPS is an ATP-dependent proteolytic system that identifies target proteins for degradation through the addition of ubiquitin (Ub) molecules to the protein. This process requires the activity of three enzymes, Ub-activating enzyme (E1), Ub-conjugating enzyme (E2), and Ub-ligase enzyme (E3). Ub is first bound to the E1 in an ATP-dependent process. Ub is then transferred from E1 to the E2 via a linkage between Ub and a cysteine residue of the E2 enzyme. Lastly, E3 catalyzes the addition of Ub to the target protein via an isopeptide bond between a lysine residue on the target protein and a glycine residue on Ub. This process is repeated until a minimum of four Ub are attached to the target protein, which is required for recognition of the target protein by the 26S proteasome. The proteasome cleaves tagged proteins into short polypeptides which additional enzymes act upon until the original protein is completely degraded (Murton, et al., 2008).

Similar to other molecular pathways, the ubiquitin-proteasome pathway appears to be influenced by certain amino acids. When a rat extensor digitorum longus was isolated and treated with solutions designed to induce proteolysis, infusion of a 10mM leucine solution decreased gene expression for two genes associated with the ubiquitin-proteasome system, resulting in significant decreases in total muscle proteolysis

(Busquets et al., 2000). In chick skeletal muscle leucine supplementation alone, as well as in combination with the other branched-chain amino acids, significantly decreased myofibrillar proteolysis both in vitro and in vivo (Nakashima, Ishida, Yamazaki, & Abe, 2005).

3-hydroxy-3-methyl-glutaryl-CoA Reductase

One of the main structural disruptions associated with exercise-induced muscle damage is the loss of sarcolemma plasma membrane integrity (Friden & Lieber, 2001). Cholesterol is a structural component of the sarcolemma making up approximately 20% of the lipid bilayer (Tortora & Derrickson, 2006) and is synthesized through the mevalonate pathway (Voet, et al., 2006). The rate limiting step in this pathway is the conversion of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) to mevalonic acid by the enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase (Voet, et al., 2006). β -Hydroxy β -methylbutyrate (HMB), a metabolite of leucine, can be converted to cytosolic HMG-CoA therefore providing substrate for the synthesis of cholesterol (Rudney, 1957). Impaired cholesterol synthesis via drug treatments affects skeletal muscle tissue, including increased muscle damage (Reijneveld, Koot, Bredman, Joles, & Bar, 1996) and decreased myoblast proliferation (van Vliet, Negre-Aminou, van Thiel, Bolhuis, & Cohen, 1996). These results suggest an important role for intracellular cholesterol synthesis in the maintenance of skeletal muscle tissue, as well as during times of repair. Leucine's catabolism to HMB may provide additional substrate for the synthesis of intracellular cholesterol required for repair of the sarcolemma following muscle damage.

Mammalian Target of Rapamycin (mTOR) Pathway

Resistance exercise that results in muscle damage has been shown to influence gene expression for proteins associated with reparative mechanisms (Chen, Hubal, Hoffman, Thompson, & Clarkson, 2003; Mahoney et al., 2008). Genes that code for various proteins have been shown to be upregulated following resistance exercise as shown by increases in mRNA content, some of which includes mechano growth factor (Hameed, Toft, Pedersen, Harridge, & Goldspink, 2008), androgen receptors (Bamman et al., 2001), and myosin heavy-chain isomers (Willoughby & Nelson, 2002). Increases in mRNA represent an increase in transcription of the subsequent genes; however, production of the functional proteins requires the mRNA to undergo translation. The translation of mRNA to protein is generally broken down into 3 main stages: initiation, elongation, and termination (Kapp & Lorsch, 2004). Processes involved in the initiation and elongation phases of translation are largely mediated through the mammalian target of rapamycin (mTOR) pathway (Wang & Proud, 2006).

The mTOR signalling pathway appears to be critical for the post natal growth of mammalian skeletal muscle, including growth which occurs following resistance exercise (Bodine, 2006). The role of mTOR on translational initiation takes place in specific regulatory steps during the initiation process, which have been reviewed thoroughly by Pain (1996). Of these regulatory steps, the one under the control of mTOR is the binding of mRNA to the 43 S preinitiation complex. The first part of this process involves the recognition and unwinding of the mRNA to allow binding to the 40 S ribosome. This step involves a group of eukaryotic initiation factor (eIF) proteins eIF4 which can also be split into two subunits, eIF4B and eIF4F (or eIF4G-eIF4E). The eIF4F complex consists

of eIF4E, eIF4A, and eIF4G. Each eIF plays an important role during the translation initiation process. eIF4A is a RNA helicase that functions with eIF4B to unwind the secondary structure in the 5'-untranslated region of the mRNA. eIF4E is a protein that binds to the m⁷GTP cap present at the 5-end of the mRNA and is important for selection and stability of the mRNA to be translated. Through association with eIF4G, eIF4E also binds to the 40 S ribosomal subunit. eIF4G provides structure and holds eIF4E, eIF4A, the mRNA and the 40S ribosomal subunit. The association between eIF4E and eIF4G is crucial for stabilizing the interaction between the mRNA and the 40S ribosome and is therefore essential for the formation of the 43 S preinitiation complex. Together the eIF4F complex collectively serves to recognize, unfold, and guide the mRNA to the 43 S preinitiation complex (Gautsch et al., 1998). One mechanism through which this process can be altered involves the binding of eIF4E to eIF4G and forming the active eIF4F complex. The availability of eIF4E for the eIF4F complex is regulated by a family of translocational repressors, the eIF4E-binding proteins (4E-BPs). 4E-BP1 competes with eIF4G for binding eIF4E and has the ability to sequester eIF4E into an inactive complex. If this occurs eIF4E cannot bind with eIF4G and therefore cannot bind with the 43 S preinitiation complex. The phosphorylation of 4E-BP1 decreases its affinity for eIF4E causing dissociation between the two, and is thought to regulate the rate of translation. The mTOR pathway is one of the main signalling pathways responsible for facilitating phosphorylation of 4E-BP1; blocking this pathway significantly decreases phosphorylation of 4E-BP1 (Anthony et al., 2000).

Another downstream target of mTOR is the ribosomal protein S6 kinase (S6K1). While the complete role of S6K1 is not fully known, S6K1 has been shown to

phosphorylate eIF4B (Raught et al., 2004), eEF2 kinase (Wang et al., 2001) and rpS6 (Roux et al., 2007). As mentioned previously, eIF4B functions as a cofactor of an RNA helicase, eIF4A, to increase its processivity (Rogers, Richter, Lima, & Merrick, 2001). eEF2 kinase negatively regulates translation elongation by phosphorylating and inhibiting eEF2, which can then be relieved through phosphorylation of eEF2 kinase by S6K1 rendering it inactive (Wang, et al., 2001). While the exact mechanisms controlled by rpS6 have yet to be identified, current investigations suggest roles in controlling cell size and glucose homeostasis, as well as regulation of protein synthesis (Ruvinsky & Meyuhas, 2006). Early investigations showed the previous substrates as the main downstream targets of S6K1, but recently other substrates have been identified as being controlled by S6K1 with each having some role in controlling cell size (Ruvinsky & Meyuhas, 2006). This has been shown when the gene for S6K1 is knocked out in mice as they exhibit significantly smaller myoblasts than mice with functional S6K1 genes (Ohanna et al., 2005).

Amino acids have been shown to be a potent stimulator of the mTOR pathway (Hara et al., 1998; Wang, Campbell, Miller, & Proud, 1998). Of all the branched-chain amino acids, leucine in particular, appears to exert the greatest effects on the mTOR pathway (Kimball & Jefferson, 2006). Vary and colleagues (1999) found that when leucine was removed from a 10x amino acid perfusate in rat skeletal muscle 4E-BP1 phosphorylation was significantly reduced, leading to increased association between eIF4G and eIF4E. Omission of leucine also resulted significantly reduced phosphorylation levels for S6K1 (Vary, et al., 1999). Similarly, oral administration of leucine significantly increased the percentage of the most highly phosphorylated form of

4E-BP1, increasing association between eIF4G and eIF4E compared to carbohydrates or placebo in the skeletal muscle of postabsorptive rats (Anthony, Anthony, et al., 2000). This activation of the mTOR pathway can also be seen following an acute bout of resistance exercise. Oral ingestion of a leucine-enriched essential amino acid and carbohydrate solution one hour following resistance exercise resulted in increased muscle protein synthesis (Dreyer et al., 2008). Results also showed significantly greater mTOR activation and phosphorylation of 4E-BP1 and S6K1, as compared to a placebo (Dreyer, et al., 2008). La Bounty, Campbell, Oetken, and Willoughby (2008) had subjects consume 60 mg/kg/bw of either leucine, 120 mg/kg/bw BCAA (providing 60 mg/kg/bw of leucine) or placebo in three equal doses 30 minutes before, immediately before, and immediately after an acute bout of lower body resistance training. Both leucine and BCAA supplementation resulted in significantly greater phosphorylation of 4E-BP1 two hours following the resistance exercise compared to the placebo, and only the BCAA group was significant after 6 hours (La Bounty, et al., 2008). These results provide evidence that ingestion of amino acids, particularly leucine, can increase protein synthesis via the mTOR pathway. Specifically, leucine activates the mTOR pathway following an acute bout of resistance training, suggesting a possible role in the recovery process.

Based on the effects of leucine on the individual cellular processes that contribute to protein degradation, synthesis, and maintenance of cellular integrity, the cumulative effect may be attenuation in the level of muscle damage and an expedited return of muscle function following eccentric exercise.

Amino Acid Supplementation and Exercise Protocols

While there are many investigations examining the effects of amino acid supplementation on physiological adaptations following resistance training, including hypertrophy (Hulmi et al., 2009; Willoughby, Stout, & Wilborn, 2007) and maximal strength (Coburn et al., 2006; Willoughby, et al., 2007), few have examined the effects on muscle damage. While the degree of muscle damage experienced during resistance training will vary depending on the protocol, supplementation remains a possible way of trying to attenuate the level of exercise-induced muscle damage. Of the macronutrients, supplementation with the full spectrum of amino acids or the essential amino acids, either alone or with carbohydrates, remains the most utilized protocols within the literature.

Protein Supplementation

Supplementation with carbohydrates and protein (6.2% carbohydrates + 1.5% protein) significantly decreased myoglobin concentrations within blood plasma 6 hours post-exercise, and creatine kinase concentrations 24 hours post-exercise after a single resistance training session (Baty et al., 2007). Nosaka, Sacco, & Mawatari (2006) reported that ingestion of a supplement containing 8 essential amino acids and 4 non-essential amino acids immediately before and in the days following a strenuous eccentric resistance training bout resulted in significantly lower levels of plasma creatine kinase and myoglobin concentrations when compared to a placebo. Consumption of a 6% carbohydrate and 6 g essential amino acid solution immediately after resistance training resulted in a significant decrease in urinary excretion of 3-methylhistidine, a direct marker of myofibrillar catabolism within skeletal muscle (Bird, Tarpenning, & Marino, 2006).

Conversely, White et al. (2008) found that supplementation with protein (23 g) and carbohydrates (75 g) either 15 minutes before or 15 minutes after exercise did not significantly decrease creatine kinase or muscle soreness after a muscle-damaging bout of eccentric contractions. Consuming a liquid supplement containing 33% protein and 67% carbohydrates did not decrease plasma creatine kinase levels after 3 days of heavy-resistance training (Kraemer, Volek, Bush, Putukian, & Sebastianelli, 1998). Chronic supplementation with either whey protein or soy protein during a 6 week resistance training program did not attenuate urinary excretion of 3-methylhistidine (Candow, Burke, Smith-Palmer, & Burke, 2006). Therefore, the role of amino acid supplementation in attenuating the levels of muscle damage associated with resistance exercise requires further investigation.

Supplementation with 25g of whey protein hydrolysate following eccentric exercise returned peak isometric torque to baseline after 6 hours, compared to a placebo which resulted in peak isometric torque being reduced up to 24 hours following exercise (Buckley et al., 2008). Supplementation during exercise with milk and a milk-based protein and carbohydrate beverage, both supplying approximately 34 g of protein each, resulted in significantly higher peak torque values 48 hours following a damaging leg flexion protocol in comparison to a placebo (Cockburn, et al., 2008). Conversely, supplementation with whey protein (23 g) and carbohydrates (75 g), either 15 minutes before or 15 minutes after exercise, was unable to attenuate the decrement in maximal voluntary contractions following eccentric resistance exercise (White, et al., 2008).

Leucine Kinetics at Rest and During Resistance Exercise

Unlike most amino acids, leucine and the other BCAAs are initially catabolised in skeletal muscle (Odessey & Goldberg, 1972). The first step in the metabolism of leucine is transamination to α -ketoisocaproate (Nissen et al., 2000). α -ketoisocaproate then undergoes oxidation in the liver to form β -hydroxy- β -methylbutyrate (HMB) via the enzyme α -ketoisocaproate dioxygenase (Sabourin & Bieber, 1983).

Resistance exercise has been shown to affect the plasma concentration of leucine. Oral ingestion of leucine significantly increases plasma concentrations of leucine with inclusion of resistance exercise significantly decreasing the rise in plasma leucine levels (Mero, Leikas, Knuutinen, Hulmi, & Kovanen, 2009). Thus, resistance exercise may also affect the metabolism of leucine to HMB.

Branched-chain Amino Acids (BCAAs)

Much of the research examining leucine supplementation during exercise is in combination with the other BCAAs, isoleucine and valine. Coombes and McNaughton (2000) had subjects supplement with 12 g of BCAAs per day or placebo for 14 days and on the 7th day of supplementation perform a 120 minute cycling bout at 70% VO₂. Following the cycling bout, CK levels were significantly lower at 2, 3, 4, 24, 72, and 120 hours post-exercise and LDH levels were significantly lower at 3, 4, 24, 72, and 120 hours post-exercise in the group that consumed BCAAs (Coombes & McNaughton, 2000). Acute ingestion of 50g of BCAAs resulted in significantly lower CK levels at 4, 24, and 48 hours following a cycling protocol of 90 minutes at 55% of V_{O2} peak (Greer, et al., 2007). Conversely, Jackman, Witard, Jeukendrup, and Tipton (2009) recently reported that acute BCAA ingestion did not attenuate serum levels of CK or MYO

following a bout of unilateral eccentric exercise. BCAA supplementation has also been shown to reduce the level of delayed-onset muscle soreness experienced 24 hours post-exercise and attenuated the decrease in leg-flexion torque 48 hours post-exercise (Greer, et al., 2007). Consuming 5 g of BCAAs 15 minutes prior to 7 sets of 20 squats significantly reduced the amount of delayed-onset muscle soreness experienced 2-5 days following the resistance exercise (Shimomura et al., 2006).

Leucine and β -Hydroxy β -methylbutyrate (HMB)

Limited literature exists on leucine supplementation alone and its effect on exercise-induced muscle damage, muscle soreness, and neuromuscular function following resistance exercise. Leucine has been shown to attenuate muscle wasting and promote protein synthesis in various catabolic states including, malnutrition (Sugawara, et al., 2007), tumor-bearing (Ventrucci, et al., 2004), and aging (Combaret, et al., 2005). Much of the research examining the effect of leucine during exercise has focused on the leucine metabolite HMB. Supplementation with 3 g of HMB/day during a 3-week resistance exercise protocol resulted in significantly lower urinary 3-methylhistidine excretion through the first 2 weeks, significantly lower CK levels at week 3, and significantly greater total body strength gains throughout all training weeks (Nissen et al., 1996). Seven weeks of supplementation with 3 g of HMB/day resulted in significantly lower plasma CK and LDH levels following a 20 km run in trained runners (Knitter, Panton, Rathmacher, Petersen, & Sharp, 2000). The effect of HMB supplementation on muscle damage following eccentric based exercise has shown conflicting results. Supplementation with 3 g/day of HMB and 0.3g/day of α -isoketocaproic acid for 14 days prior to performing an elbow flexion damage protocol resulted in an attenuated CK

response and percent decrement in 1RM strength in the 72 hours following the exercise (van Someren, Edwards, & Howatson, 2005). However, when an identical supplement protocol was applied to a downhill running model, there was no effect on CK response, isometric and isokinetic torque, or muscle soreness (Nunan, Howatson, & van Someren, 2010). With the amount of literature that shows that leucine supplementation may help preserve skeletal muscle during various catabolic states, it remains unclear whether leucine is a viable supplement to attenuate the damage response brought about by eccentric exercise.

METHODOLOGY

Subjects

A total of 27 healthy, college-aged male subjects were recruited for this investigation (height 178.62 ± 5.54 cm; body mass 77.71 ± 13.46 kg; age 21.26 ± 1.56 years). Subjects were excluded based on a) participation in a structured lower-body resistance exercise program in the 6 months prior to the investigation, b) use of ergogenic aids in the previous three months, c) or if currently taking any prescription drugs that impair cholesterol synthesis or reduce inflammation. Prior to initial testing, subjects were required to read and sign an informed consent which was approved by the Institutional Review Board at Appalachian State University.

Study Design

The study was a double-blind, placebo controlled study design consisting of two supplement groups ($n=10$, $n=9$) and a control group ($n=8$). Subjects were randomly assigned to their respective groups. Subjects were asked to refrain from performing any type of resistance exercise or strenuous activity 48 hours prior to each testing session and in the 96 hours following each testing session. The first testing session consisted of collecting anthropometric measurements (height, weight, age), obtaining baseline values for muscle function, and determining each subject's one repetition maximum (1RM) in the leg press exercise (Table 1). During the second testing session, the subjects performed an eccentric-based resistance exercise protocol in which three doses of the

supplement was consumed before, during and after the exercise protocol. The control group did not perform the resistance exercise protocol nor ingest any form of supplement. Subjects then reported for post-testing on the muscle function tests at 24, 48, 72 and 96 hours following the resistance exercise protocol. Subjects were instructed to refrain from taking any type of non-steroidal anti-inflammatory drugs (NSAIDs) (e.g. ibuprofen, Advil™) during the duration of the study. Subjects reported to the laboratory in a fasted state for the resistance exercise session and for all of the post-testing sessions. Prior to performing the resistance exercise, subjects were given a standardized meal. Blood samples were taken at pre-exercise, 24, 48, 72, and 96 hours post-exercise and analyzed for indices of muscle damage. Measures of muscle function and perceived muscle soreness were obtained at the pre-exercise and 24, 48, 72, 96 hours post-exercise time points. Muscle function was assessed using a maximal isometric squat, as well as various maximal vertical jumps. Perceived muscle soreness was assessed via a subjective delayed-onset muscle soreness scale. Subjects were instructed to keep dietary records for the 48 hours prior to the resistance exercise session and in the 72 hours following the resistance exercise session. Dietary records were analyzed for total caloric intake, as well for quantity of each macronutrient (carbohydrates, protein, and fat).

Muscle Function Assessment

During the first testing session, subjects performed three maximal isometric squats, as well as three of each of the following jumps; static jump (SJ), countermovement jump (CMJ), drop jump from 20cm (DJ20), drop jump from 40cm (DJ40), and drop jump from 60cm (DJ60). The initial order with which the jumps were performed was determined randomly for each subject and then held consistent during

each of the post-exercise testing sessions. Subjects performed the same tests again 24, 48, 72 and 96 hours following the resistance exercise protocol, however during these session only two trials for each test were performed in order to minimize accumulating fatigue and muscle damage. Subjects were encouraged to give maximal effort during each of the jumps, and adequate rest was giving between each jump trial.

Each isometric squat was performed with the subject standing on a force plate (BP6001200, AMTI, Watertown, MA) with a fixed-position barbell placed across their upper back. Subjects were instructed to exert maximal force against the bar for 3 seconds. All isometric squats were performed with a knee angle of 100 degrees.

All vertical jump testing was performed with the subject standing on a force plate while holding a weightless (plastic) bar across their upper back. The right side of the barbell was attached to two linear position transducers (LPTs) (PT5A-150, Celesco Transducer Products, Chatsworth, CA). The weightless bar acted to counterweight the pull of the two LPTs resulting in zero load. The LPTs were located above-anterior and above-posterior to the subject and, when attached to the bar, resulted in the formation of a triangle. This allowed for the calculation of vertical and horizontal displacements through trigonometry involving constants and displacement measurements. This method of collecting kinematic variables has previously been validated (Cormie, McBride, & McCaulley, 2007). The combined retraction tension of the LPTs was 16.4 N; this was accounted for in all calculations. Analog signals from the force plate and LPTs were collected for every trial at 1000 Hz using a BNC-2010 interface box with an analog-to-digital card (NI PCI-6014, National Instruments, Austin, TX). Custom programs

designed using LabVIEW (Version 8.2, National Instruments) were used for recording and analyzing the data.

1 RM Testing

A warm-up protocol will consist of loads equal to 30% (8-10 repetitions), 50% (4-6 repetitions), 70% (2-4 repetitions) and 90% (1 repetition) of an estimated 1 RM. Subjects were given up to four maximal attempts to achieve their 1RM. During all leg press attempts, subjects were required to lower the weight to a point where an 80 degree knee angle was attained. Rest periods of 3 to 5 minutes were given between trials. Any necessary modifications during testing were determined by a Certified Strength and Conditioning Specialist.

Resistance Exercise Protocol

The resistance exercise protocol emphasized the eccentric component of each exercise as eccentric muscle contractions have shown to induce the highest levels of muscle damage (Gibala, et al., 1995). The resistance exercise protocol was modified from damage protocols previous utilized by Miyama and Nosaka (2004) and Cooke et al. (2009). Both protocols were shown to induce significant levels of muscle damage as shown through increases in plasma creatine kinase. Subjects were instructed to perform a 5 minute warm-up on a cycle ergometer. The exercise protocol consisted of 5 sets of 20 maximal drop jumps from a height of 60 cm with a 10 second interval between jumps and a 2 minute rest between sets. Subjects then performed 6 sets of 10 eccentric contractions on the leg press with a weight equal to 120% of their concentric 1 RM. During all repetitions, the eccentric portion of the movement was performed with a 3 second tempo.

If the desired tempo could not be maintained due to fatigue, subjects were allowed a 10-15 second rest before continuing with the set. A 3 minute rest was given between sets.

Supplement Protocol

Supplement groups included a placebo (P) group (n=9) and a leucine (L) group (n=10). The control (C) group (n=8) did not get any form of supplement. Leucine supplementation consisted of each dose containing 250 mg/kg body weight of L-leucine. Additionally, 3g of non-caloric sweetener (Splenda[®]) was added to the supplement. Doses were provided 30 minutes prior to resistance exercise, immediately pre-exercise, immediately post-exercise, and at the beginning of each of the post-testing sessions (24, 48, 72 and 96 hours post-exercise). P consisted of Splenda[®] alone at all time points. Each dose for both the L and P groups were mixed with a liquid solution containing 2 g of a low-calorie flavoring mixture (Crystal Light[®]) to increase palatability of the supplement protocols. Each dose was mixed in a shaker bottle with additional water being added following initial consumption to ensure the entire supplement was ingested and none remained in the bottle. Following oral ingestion of leucine, peak concentrations of plasma leucine occur at 67 minutes (Mero, Leikas, Knuutinen, Hulmi, & Kovanen, 2009).

Blood Collection

Blood was collected pre-exercise, 24, 48, 72 and 96 hours post-exercise. The resting values were used to determine baseline levels for muscle damage markers. Blood was obtained from the antecubital vein into a 10 ml Vacutainer[™] blood collection tube and allowed to clot at room temperature. The whole blood was centrifuged for 15 minutes at 2500 rpm at room temperature with the serum being divided into Eppendorf[™] tubes and frozen at -80°C for subsequent analysis.

Biochemical Analysis

Serum from the pre-exercise, 24, 48, 72 and 96 hours blood samples were analyzed for CK, LDH, and MYO. CK was analyzed in duplicate using basic spectrophotometric techniques (Pointe Scientific, Canton, MI). Reagent was reconstituted with distilled water. 1000 μ l of reagent was pipetted into a polystyrene cuvette and pre-warmed at 37°C for five minutes. Twenty-five microliters of sample was added to the reagent and incubated at 37°C for two minutes. Following the incubation period, absorbance at 340 nm was determined at three time points each separated by one minute using a spectrophotometer (Genesys 5, Thermo Spectronic, Rochester, NY). Average absorbance difference per minute was calculated and then multiplied by the factor 6592 to yield results in U/L. If the calculated concentration was above 1500 U/L the sample was diluted 1:1 with saline and re-analyzed.

LDH was analyzed in duplicate using basic spectrophotometric techniques (Pointe Scientific, Canton, MI). Reagent was reconstituted with distilled water. 1000 μ l of reagent was pipetted into a polystyrene cuvette and pre-warmed at 37°C for five minutes. Twenty-five microliters of sample was added to the reagent and incubated at 37°C for one minute. Following the incubation period, absorbance at 340 nm was determined at two time points separated by one minute using a spectrophotometer (Genesys 5, Thermo Spectronic, Rochester, NY). The change in absorbance was calculated and then multiplied by the factor 6592 to yield results in IU/L.

MYO was analyzed in duplicate using a solid phase enzyme-linked immunosorbent assays (DRG International Inc., Mountainside, NJ). Serum was diluted 10 fold and then 20 μ l of diluted sample was pipetted into the appropriate well. 200 μ l of

Enzyme Conjugate Reagent was added to each well and then mixed for 30 seconds. The plate was incubated at room temperature for 45 minutes and then each well was rinsed five times with distilled water. 100 μ l of TMB reagent was added to each well and incubated at room temperature for 20 minutes. The reaction was stopped by adding 100 μ l of Stop Solution to each well. The absorbance of each well was determined by using a microplate spectrophotometer (μ Quant, Bio-Tek Instruments, Inc., Winooski, VT) at a wavelength of 450 nm.

Muscle Function Analysis

Signals from the two LPTs and the force plate underwent rectangular smoothing with a moving average half-width of 12. A force-time curve was calculated for each isometric squat. Peak isometric force (ISOPF) was measured as the highest force output achieved during the 3 second isometric contraction. Displacement-time and force-time curves were calculated for each vertical jump. Peak concentric force (PCF) and jump height (JH) were measured during the concentric phase of the vertical jump for each trial (SJ, CMJ, DJ20, DJ40, DJ60). Additionally, peak impact force (PIF) was calculated for the 40 cm and 60 cm drop jumps and peak amortization force (PAF) was calculated for each of the drop jumps. PCF was measured as the maximum force reached during the concentric phase. PIF was measured as the force generated upon contact with the force plate. PAF was measured as the force during the amortization phase of the jump between the eccentric and concentric phases. JH was determined as the difference between maximum displacement reached during the jump and initial displacement while in a standing position. The jump trial which produced the highest JH was used to determine

the values for the additional variables to be used for statistical analysis. Changes across time were expressed as a percentage change from the PRE value.

Perceived Muscle Soreness

Subjects perceived muscle soreness was assessed using a delayed-onset muscle soreness scale prior to blood collection at pre-exercise, and 24, 48, 72 and 96 hours post-exercise (Buford et al., 2009). Subjects subjectively rated their level of soreness by drawing an intersecting line across the continuum line extending from 0 cm (0 = no soreness) to 13 cm (13 = extreme soreness) (Figure 1). The distance of each mark was measured from zero with the corresponding value being used as the perceived level of muscle soreness.

Dietary Analysis

Subject's dietary intake was not standardized for this investigation; however subjects were instructed to maintain their normal dietary habits for the duration of the study. Subjects in the L and P group were instructed to keep a 6-day food log which began 48 hours prior to the resistance exercise protocol and was continued until the final post-testing session. The dietary food logs were evaluated using a dietary assessment food software program (Food Processor® SQL, ESHA Research, Salem, OR) to determine the average daily caloric (CAL) intake and macronutrient breakdown of carbohydrates (CHO), protein (PRO) and fat (FAT).

Statistical Analyses

All statistical analyses were performed on SPSS version 16.0 (SPSS Inc., Chicago, Illinois, USA). To determine whether differences existed between the groups at the PRE time point a one-way analysis of variance (ANOVA) was performed between groups. If

significant differences existed, post hoc analysis was performed using a Bonferroni test if the Levene's test was $p > 0.05$ and a Dunnett C test if the Levene's test was $p \leq 0.05$.

Between group and within group differences for all of the biochemical and biomechanical variables were determined using a general linear model with repeated measures. The PRE time point was used as a covariate if a significant difference existed between the groups at PRE. If significance was found, independent t-tests were used to determine between group differences at each time point and paired t-tests were used to determine differences across time points when compared to the PRE value. Analysis of dietary intake was done using independent t-tests for each of the variables. For all statistical measures significance was set at $p \leq 0.05$.

RESULTS

Biochemical

CK was significantly elevated in the P group at the 72 and 96 hour time points (Figure 2). CK was elevated at the 24 h time point for the L group ($p=0.06$) and the 24 and 48 hour time points for the P group ($p=0.06$ and 0.07 , respectively) but did not reach statistical significance. CK significantly decreased in the C group at the 24 hour time point. The L group had significantly higher CK levels when compared to the C group at the 24 hour time point. There was no change in LDH levels across time for any of the groups (Figure 3) and no between group differences at any of the time points. MYO was significantly elevated in the P group at the 96 hour time point (Figure 4). MYO for the L group was significantly different from the P group at PRE and 72 hours and the C group at 96 hours. The P group was significantly different from the C group at PRE.

Muscle Function

Isometric Squat

Peak isometric force decreased significantly for both the L and P groups 24, 48 and 72 hours post-exercise compared to the PRE value (Figure 5). Peak isometric force was still significantly decreased at 96 hours for the P group. When compared to the C group, L and P were significantly different at the 24, 48 and 72 hour time points with the P group also significantly different at the 96 hour time point.

Jump Height

SJ JH was significantly different for the P group at the 24 and 48 hour time points when compared to the C group (Figure 6). The P group SJ JH was significantly lower than their pre-exercise value at all time points. CMJ JH for the L group significantly decreased at the 48, 72 and 96 hour time points when compared to PRE (Figure 7). CMJ JH for the P group was significantly lower than their PRE value at all time points. CMJ JH for the L and P group was not different than the C group at any time points. DJ20 JH for the L and P groups significantly decreased across all time points compared to PRE (Table 2). DJ20 JH for the L and P group was significantly lower than the C group at the 24, 48 and 72 hour time points. DJ40 JH for the L group significantly decreased from PRE at 24 and 48 hours (Table 3). DJ40 JH for the P group significantly decreased from PRE at all time points and was significantly lower than the C group at 24 and 48 hours. DJ40 JH for the P group significantly decreased from PRE at 24, 72 and 96 hours. DJ60 JH for the L group significantly decreased from PRE across all time points and was significantly lower than the control group at the 24 hour time point (Table 4). DJ60 JH for the P group significantly decreased from PRE at the 24, 48 and 72 hour time points.

Peak Concentric Force

SJ PCF significantly increased from PRE in the L group at the 48, 72, and 96 hour time point (Figure 8). SJ PCF for the L group was higher than the P group at the 24 and 48 hour time points. CMJ PCF for the L group significantly increased from PRE at the 48 hour time point (Figure 9). DJ20 PCF for the P group significantly increased from PRE at the 48, 72 and 96 hour time points (Table 2). DJ40 PCF for the C group significantly decreased at the 24 and 48 hour time points when compared to PRE (Table 3)

but there were no significant differences between any of the groups at any of the time points. DJ60 PCF for the L group significantly increased from PRE at the 72 hour time point (Table 4).

Peak Impact Force

DJ40 PIF for the L group significantly increased from PRE at the 24, 48 and 72 hour time points (Table 3). DJ40 PIF for the L group was significantly higher than the C group at the 24 and 72 hour time points. DJ60 PIF for the L group significantly increased from PRE at the 24, 48 and 72 hour time points (Table 4). DJ40 PIF for the L group was significantly higher than the C group at the 24 hour time point.

Peak Amortization Force

DJ20 PAF for the L and P group significantly decreased from PRE at the 24 hour time point with the L group still being significantly lower at the 48 and 72 hour time points (Table 2). DJ20 PAF for the L group was significantly lower than the C group at the 48 and 72 hour time points. DJ40 PAF for the L and P group significantly decreased from PRE at the 24, 48 and 72 hour time point with the P group still being significantly lower at the 96 hour time point (Table 3). DJ40 PAF for the L group was significantly lower than the C group at the 24 and 48 hour time points. DJ40 PAF for the P group was significantly lower than the C group at the 24, 48 and 72 hour time points. DJ60 PAF for the P group was significantly decreased at the 24 hour time point when compared to PRE. DJ60 PAF for the L group was significantly higher than the P group at the 24 hour time point (Table 4). DJ60 PAF for the P group was significantly lower than the C group at the 24 and 96 hour time points.

Perceived Muscle Soreness

PMS for the L and P group significantly increased following exercise at the 24, 48 and 72 hour time points with the L group still being significantly higher at the 96 hour time point (Figure 10). PMS for the L group was significantly different from the P group at the 48 hour time point. PMS for the L and P group was significantly different from the C group at the 24, 48 and 72 hour time points with the L group still being significantly different at the 96 hour time point.

Dietary Analysis

There were no significant differences between the L and P groups for average CAL or average intake of CHO, PRO or FAT. There was also no significant difference between groups for any single day during the experimental protocol. Average values for each group are shown in Table 5.

DISCUSSION

The primary finding of this investigation is that leucine supplementation has no effect on indices of muscle damage following eccentric-based resistance exercise. An increase in CK and MYO following the exercise protocol was observed in the L and P groups at various time points; however LDH showed no deviation from pre-exercise levels. Supplementation with leucine may provide a protective effect against the loss of muscle function and vertical jump performance following eccentric exercise mainly through maintenance in force output. Performance measures in muscle contractions that were preceded by a stretch-shorten cycle were less affected by the damage protocol when compared to SJ. However, they showed no differences between supplementation protocols. Due to inconsistencies between the muscle damage markers and muscle function, effectiveness of leucine on increasing recovery following exercise-induced muscle damage remains unclear.

Assessment of damage to contractile proteins was outside the scope of this investigation, therefore serum CK and MYO levels were used to assess the levels of muscle damage. The resistance exercise protocol elicited an increase in serum levels of both CK and MYO. Due to their presence within the sarcolemma, both of these damage markers provide an indirect method of evaluating the level of muscle damage. In some instances elevated CK accompanies structural protein damage (Martinez-Amat et al., 2005), although this is not always the case (Sorichter, Puschendorf, & Mair, 1999).

Release of these molecules into the serum would indicate plasma membrane disruption which was one of the mechanisms through which leucine and metabolites are believed to reduce muscle damage (Nissen, et al., 2000).

CK elevated post-exercise in both groups, however the level of response varied greatly between subjects. This is a common occurrence (Baty, et al., 2007; Cockburn, et al., 2008; White, et al., 2008) and creates problems when trying to determine group differences. The logical choice would be to use a crossover design, but this is not always possible when trying to induce muscle damage because of the repeated-bout effect (Ebbeling & Clarkson, 1989). CK was only significantly elevated in the P group at the 72 and 96 hour time point. Yet, there were no significant between group differences, which would indicate that leucine has no effect on attenuating muscle damage. These results refute existing literature that showed an attenuated CK response following three weeks of resistance exercise in individuals supplementing with HMB (Nissen, et al., 1996). Similarly, acute supplementation of 50 g of BCAAs, providing 25 g of leucine, was able to attenuate the CK response following a 90 minute cycling protocol at 55% VO_2 maximum (Greer, et al., 2007). This discrepancy may be due to the type of exercise protocols used to elicit a damage response. Both of the previous investigations had a much lower CK response than that of the current investigation. Consequently, if the tissue damage was too extensive, leucine may have been unable to repair such a high level of damage. Using a similar muscle damage and supplementation protocol to the current investigation, Jackman et al. (2009) reported that consuming BCAA's during and in the days following unilateral eccentric exercise had no effect on CK levels.

It is unclear why LDH levels did not increase following the exercise protocol. Serum sample storage at -80°C could have inactivated some of the thermolabile isoenzymes (Kreutzer & Fennis, 1964). However, sample storage at -90°C for 90 days was shown to not alter LDH activity (Shain, Boesel, Klipper, & Lancaster, 1983). Previously, Cooke et al. (2009) utilized a similar resistance protocol and found a significant increase in LDH activity 24, 48, 72 and 96 hours post-exercise. However, the previous investigation also reported CK values 20 times that of the current investigation, indicating that their protocol elicited a much greater damage response (Cooke, et al., 2009). Therefore, the current investigation may not have produced a great enough damage response to see a significant change in LDH.

MYO followed a similar response pattern to CK which supports what has been reported by previous investigations (Beck et al., 2007; Cockburn, et al., 2008). Interestingly, there was less of an effect by the exercise protocol on myoglobin concentrations, as number of time points above pre-exercise values was less than what was observed by CK. While there was less within group differences, there were more significant between group differences than what was seen with CK. The L group had higher MYO concentrations than the P group at the 72 hour time point. These results indicate at the L group may have actually sustained more muscle damage than the P group. The physiological reason underlying why the L group displayed higher levels of markers for muscle damage cannot be determined by the current variables that were examined.

Following the resistance exercise protocol, there was a significant decrease in isometric squat PF output. The decrement was the greatest at the 24 hour time point and

then returned towards baseline during the recovery days. This pattern of recovery is similar to what has been observed with single-joint isometric force (Byrne & Eston, 2002b). No investigations have utilized a multi-joint exercise to determine isometric muscle function, which is most likely because most muscle damage protocols are single-joint unilateral protocols. Leucine supplementation showed no effect between experimental groups; however the L group was not significantly different from their pre-exercise value nor significantly different from the C group at the 96 hour time point. These findings are similar to those by Paddon-Jones, Keech and Jenkins (2001) who found that short-term HMB supplementation showed no effect on elbow-flexion peak torque when compared to placebo. However, contrary to the previous study, leucine supplementation was able to return isometric PF to baseline after 96 hours. This pattern for a more rapid return of muscle function to pre-exercise values was observed with a combined HMB and α -isoketocaproic acid supplement protocol (Nunan, et al., 2010).

While there is existing literature showing the effect of muscle damage on dynamic muscle contractions (Byrne & Eston, 2002a; Marginson, Rowlands, Gleeson, & Eston, 2005) there is no literature which looks at amino acid supplementation and its effects on jumping performance following muscle damage. Exercise-induced muscle damage has been shown to significantly impair SJ jump performance for up to three days following the exercise (Byrne & Eston, 2002a); however leucine supplementation was unable to attenuate the decrease in SJ performance when compared to the placebo. Despite the absence of between group differences, the L groups SJ JH did not show any significant decrease from their pre-exercise JH. Due to the static starting position prior to initiation of the concentric phase, there is greater reliance on the contractile apparatus of the

muscle to initiate the movement. If there was less damage to the contractile components within the muscle then the muscle would maintain a higher degree of functionality. Since actual disruption of the sarcomeres was not examined in the current study it is unclear to what degree the contractile structures were damaged. Still, PF during the SJ was significantly higher in the L group at the 24 and 48 hour time points when compared to the P group. The L groups PF values were similar to those of the C group who did not sustain any muscle damage. Previous literature has shown a significant decrease in PF output in the SJ following muscle damage (Garcia-Lopez, et al., 2006), which supports what was found in the P group but in contrary to what was observed in the L group. It is unclear as to why the PF significantly increased during the recovery days in the L group, however a similar recovery pattern was observed in the C group so a partial explanation may be familiarization with the testing procedures. It can be speculated that maintenance of PF output may indicate that the contractile apparatus was less damaged in the L group, because force output of the muscle relies on the integrity of the contractile apparatus.

Unlike the isometric and concentric-only muscle contractions, the muscle contractions preceded by an eccentric phase showed no clear similarities between experimental groups. This may be due to those contractions utilizing the stretch-shorten cycle which has been shown to be less affected by exercise-induced muscle damage (Byrne & Eston, 2002a; Harrison & Gaffney, 2004). JH significantly decreased during the CMJ, DJ20, DJ40, and DJ60 for both experimental groups. The time-course of recovery back to baseline varied slightly with jumping condition. The L group recovered faster for the DJ40 and the P group recovered faster for the CMJ and DJ60. The reason underlying the discrepancy between the two groups is unclear.

One of the novel aspects of this investigation was examining the various force outputs during the different phases of the drop jumps. Due to the nature of drop jumps, there is an increased muscular component placed on the eccentric phase of the jump as seen by an increase in muscle activity measured by electromyography (McBride, McCaulley, & Cormie, 2008). This increased muscle activation is required in order to maintain muscle length and stiffness during the overloaded eccentric phase of the jump, and is required for optimal performance in the drop jump (Horita, Komi, Nicol, & Kyrolainen, 2002). Although stiffness of the muscle was not measured, the forces applied during landing significantly changed following exercise. PIF increased in both the L and P groups, however only the L group showed a significant increase. Nevertheless, this increase in PIF did not correspond to an increase in DJ performance. Certain time points which had the highest PIF had the lowest JH. The changes in PIF followed a similar trend to the PMS which may explain the increase in PIF. If the subject had a high level of muscle soreness they may have been reluctant to absorb the energy generated from the initial drop, which would therefore increase the PIF generated during landing.

Since muscle function was compromised, there was a decrease in force output during the amortization phase of the jump across all three drop jump conditions. For the DJ40 and DJ60, the L group was able to maintain a higher amortization force output than the P group. The L group returned to baseline at the 96 hour time point for the DJ40 PAF and had a significantly higher PAF at the 24 hour time point for DJ60. For the DJ20, the P group returned to baseline faster than the L group which does not support the results of the other two drop jump conditions. This may be due to the fact that the eccentric phase

of the DJ20 trials was not overloaded to the same degree as the higher drop jumps. Therefore, it could be reasoned that a decreased level of muscle function would not impact the amortization phase of the DJ20 to the same degree as the other two drop jump types.

As expected, PMS significantly increased following the resistance exercise in both experimental groups. Surprisingly, the L group had significantly higher PMS than the P group at the 48 hour time point. This conflicts with previous investigations that found that PMS was lower following eccentric exercise (Jackman, et al., 2009) and traditional resistance exercise (Shimomura, et al., 2006) when subjects ingested leucine along with the other BCAAs. However, the current results partially support previous investigations that showed no effect of leucine metabolite supplementation on PMS following downhill running (Nunan, et al., 2010) or eccentric resistance exercise (Paddon-Jones, et al., 2001). The factors contributing to the L group experiencing greater muscle soreness remain unclear. One explanation may be an alteration in the inflammatory response as a result of leucine supplementation (Bassit et al., 2002). Increased inflammation is thought to be a major contributing factor to muscle soreness (Smith, 1991), however recent investigations show that they do not always positively correlate (Paulsen et al., 2010).

This investigation was the first to look at the effect of leucine supplementation on indices of muscle damage and the time course of muscle recovery following eccentric-based resistance exercise. There was a minimal effect between leucine supplementation when compared to placebo for all the dependent variables. Based on these results, leucine does not appear to be a viable option at attenuating muscle damage and may

actually increase muscle damage and perceived muscle soreness two days following resistance exercise. However, in some instances leucine supplementation was able to maintain force output following the resistance exercise. Additional investigations need to be conducted to determine if leucine supplementation should be used when attempting to recover from a resistance exercise bout. Utilization of a resistance exercise model that induces damage levels comparable to those in BCAA supplementation studies may be able to determine whether leucine can increase recovery following lower levels of damage.

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varying levels of age, sex, and training experience: A review. *Nutrition and Metabolism (London)*, 5, 1.

Table 1. Anthropometric and strength values for each group (Mean±SD).

Group	Height (cm)	Body Mass (kg)	Age (yrs)	Leg Press 1RM (kg)
Leucine	178.45±6.60	76.79±12.47	21.00±1.05	206.58±53.79
Placebo	178.71±5.99	79.59±14.65	21.67±1.94	218.37±70.06
Control	178.74±4.08	76.76±14.84	21.13±1.73	209.47±95.62

Table 2. Biomechanical variables (Mean±SD) during the DJ20 following the resistance exercise protocol. * denotes significantly different from PRE and C group. † denotes significantly different from PRE.

Group	Time	JH (m)	PCF (N)	PAF (N)
Leucine	PRE	0.439±0.034	1634.23±408.58	2020.36±341.51
	24h	0.394±0.059*	1668.29±477.69	1770.55±270.76†
	48h	0.391±0.039*	1678.42±409.52	1752.45±323.60*
	72h	0.396±0.042*	1689.22±364.00	1718.35±311.51*
	96h	0.401±0.058†	1620.22±343.45	1808.75±341.76
	PRE	0.481±0.040	1679.18±371.93	2049.28±438.33
Placebo	24h	0.420±0.042*	1741.39±394.99	1791.24±369.26†
	48h	0.430±0.048*	1788.05±369.03†	1832.64±375.58
	72h	0.435±0.061*	1777.24±406.93†	1881.35±409.53
	96h	0.443±0.052†	1800.58±435.06†	1892.04±480.62
	PRE	0.434±0.050	1659.15±270.02	2055.73±883.67
	24h	0.444±0.043	1677.28±270.95	2007.22±866.74
Control	48h	0.431±0.034	1697.24±351.33	2034.09±701.05
	72h	0.431±0.037	1656.27±254.58	2031.45±732.89
	96h	0.425±0.030	1722.99±393.79	2041.11±725.48
	PRE	0.434±0.050	1659.15±270.02	2055.73±883.67

Table 3. Biomechanical variables (Mean±SD) during the DJ40 following the resistance exercise protocol. * denotes significantly different from PRE and C group. † denotes significantly different from PRE.

Group	Time	JH (m)	PCF (N)	PIF (N)	PAF (N)
Leucine	PRE	0.434±0.047	1633.07±308.50	1990.24±645.48	1965.01±315.34
	24h	0.400±0.059†	1652.41±351.93	2195.86±512.04*	1748.92±294.09*
	48h	0.385±0.063†	1637.51±321.44	2365.66±564.15†	1738.13±321.66*
	72h	0.401±0.066	1679.15±356.84	2299.55±543.54*	1778.21±365.15†
	96h	0.415±0.064	1662.99±339.27	2086.92±531.27	1848.38±373.99
	PRE	0.503±0.050	1700.02±376.93	2108.10±634.05	2206.26±411.85
Placebo	24h	0.440±0.057*	1676.89±321.02	2210.55±578.29	1870.05±405.04*
	48h	0.437±0.072*	1793.52±347.19	2273.62±658.02	1793.52±347.18*
	72h	0.439±0.054†	1755.54±378.27	2190.27±582.46	1838.28±355.02*
	96h	0.448±0.054†	1790.27±442.29	2253.16±644.61	1858.60±465.76†
	PRE	0.459±0.035	1639.89±212.23	2094.91±441.67	2088.06±857.51
	24h	0.439±0.035†	1588.84±202.49†	1902.41±292.16	2096.21±967.39
Control	48h	0.439±0.055	1583.92±239.17†	1962.85±334.82	2090.31±873.76
	72h	0.427±0.056†	1617.83±214.93	1979.80±296.24	2015.87±797.89
	96h	0.426±0.042†	1653.08±256.43	2099.88±548.13	1989.80±699.00
	PRE	0.459±0.035	1639.89±212.23	2094.91±441.67	2088.06±857.51

Table 4. Biomechanical variables (Mean±SD) during the DJ60 following the resistance exercise protocol. * denotes significantly different from PRE and C group. \$ denotes significantly different from P group. # denotes significantly different from C group † denotes significantly different from PRE.

Group	Time	JH (m)	PCF (N)	PIF (N)	PAF (N)
Leucine	PRE	0.457±0.051	1593.94±315.17	2529.10±517.44	1905.19±315.17
	24h	0.400±0.075*	1575.90±297.88	2841.35±652.36*	1881.84±284.60\$
	48h	0.412±0.058†	1662.28±331.83	3060.61±818.85†	1776.24±277.92
	72h	0.420±0.052†	1695.14±358.36†	2980.45±841.98†	1766.70±281.27
	96h	0.410±0.046†	1617.09±284.27	2780.19±663.96	1828.30±313.21
	PRE	0.494±0.054	1684.27±335.99	2896.43±962.91	2102.56±396.50
Placebo	24h	0.453±0.066†	1641.11±319.40	3002.68±807.64	1852.95±490.32*
	48h	0.451±0.058†	1677.22±292.19	3167.17±942.85	1930.73±439.23
	72h	0.447±0.057†	1706.56±304.62	2963.29±912.48	2017.62±475.57
	96h	0.468±0.054	1745.00±396.08	3016.47±861.96	1932.01±434.54#
	PRE	0.459±0.056	1614.70±208.47	2686.76±528.10	1979.45±1001.9
	24h	0.454±0.045	1601.99±231.78	2624.28±530.55	2068.56±1100.7
Control	48h	0.441±0.039	1614.94±243.86	2845.57±631.91	1984.50±810.40
	72h	0.441±0.038	1611.12±251.93	2680.29±568.90	2056.71±883.73
	96h	0.443±0.036	1617.71±271.41	2842.80±643.79	2054.79±865.44

Table 5. Average daily intake of total calories, carbohydrates, protein and fat determined by a 6-day food recall (Mean±SD).

Group	CAL (kcal)	CHO (g)	PRO (g)	Fat (g)
Leucine	1822.6±573.3	228.2±59.7	72.8±25.0	64.6±34.1
Placebo	1869.5±888.8	240.3±109.4	72.5±21.0	56.9±24.5

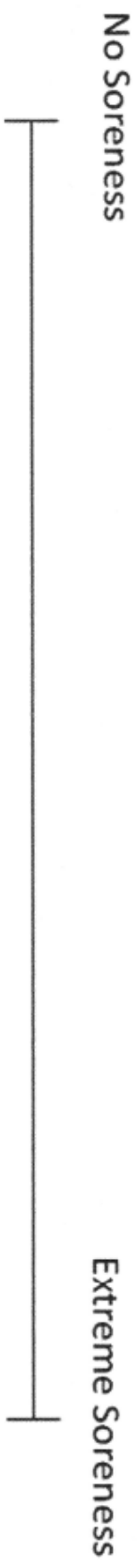


Figure 1. Perceived Muscle Soreness Scale.

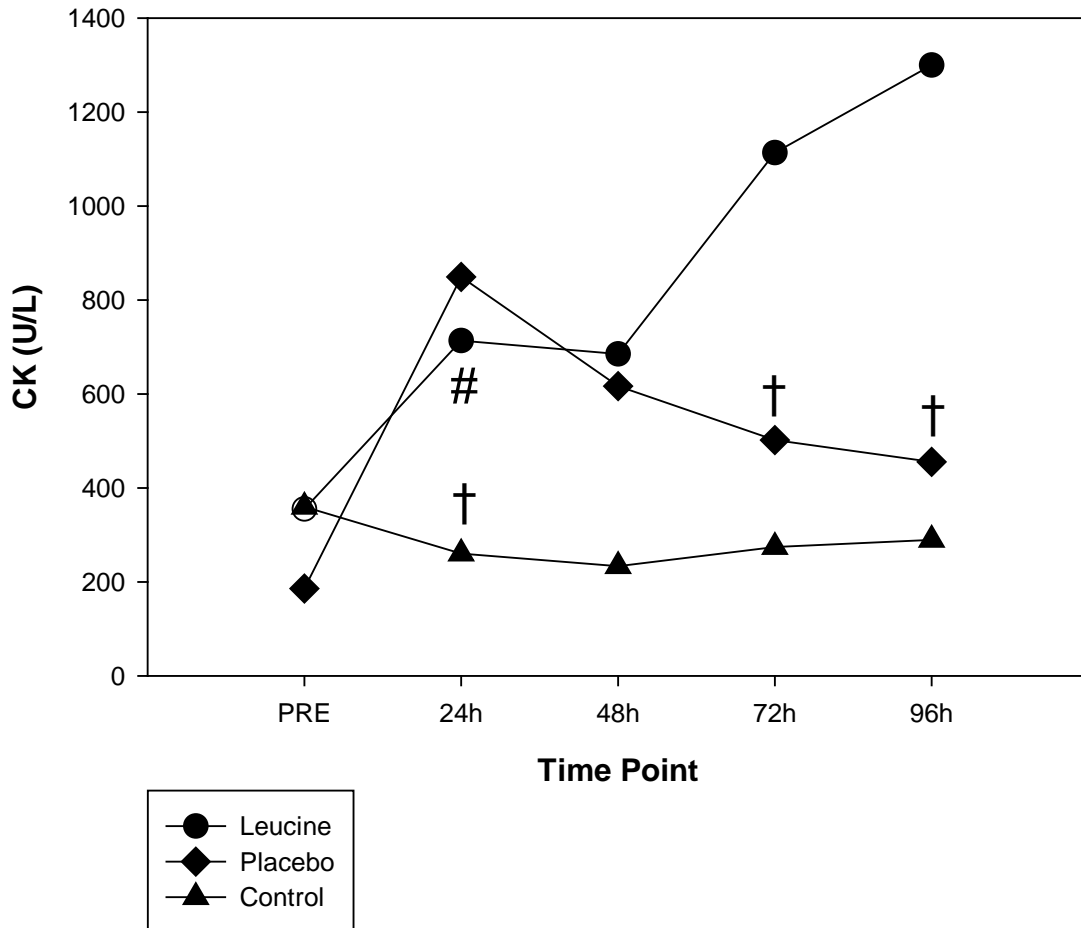


Figure 2. Creatine kinase activity following the resistance exercise protocol. # denotes significantly different from C group. † denotes significantly different from PRE.

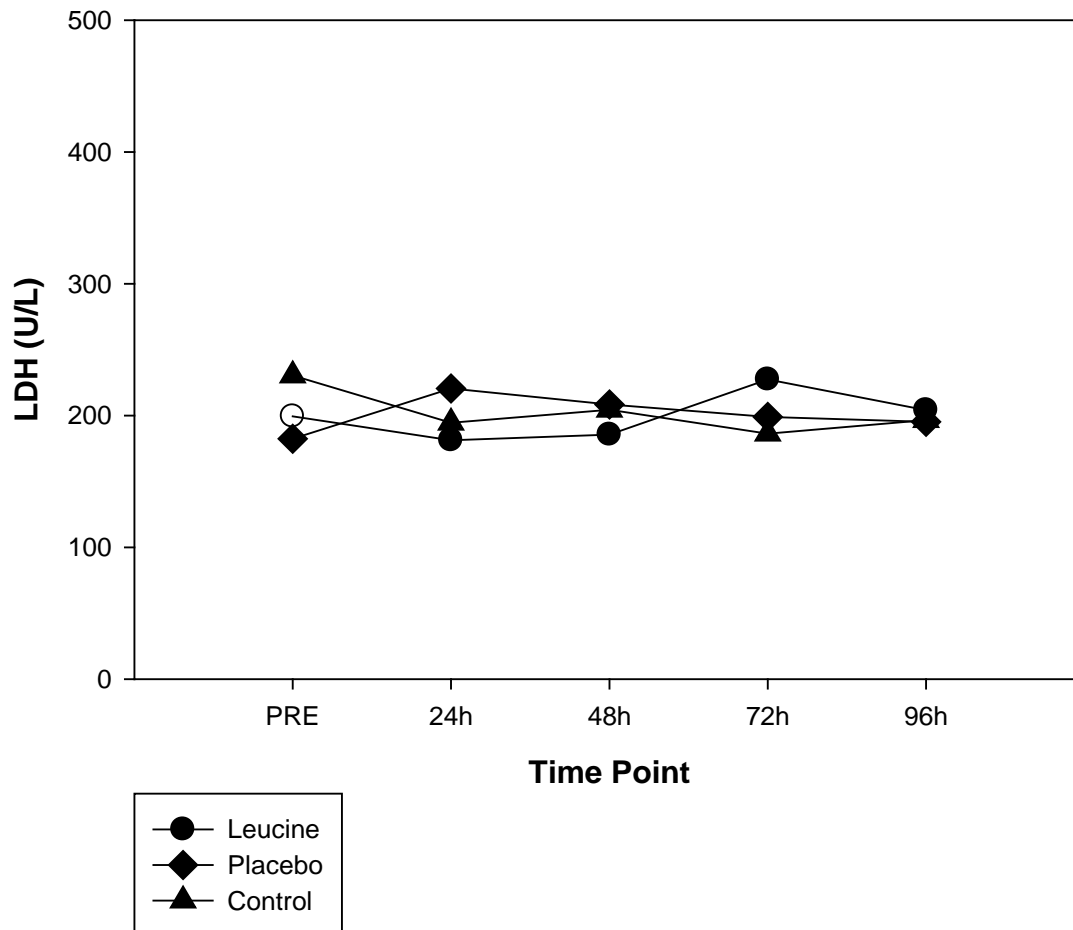


Figure 3. Lactate dehydrogenase activity following the resistance exercise protocol.

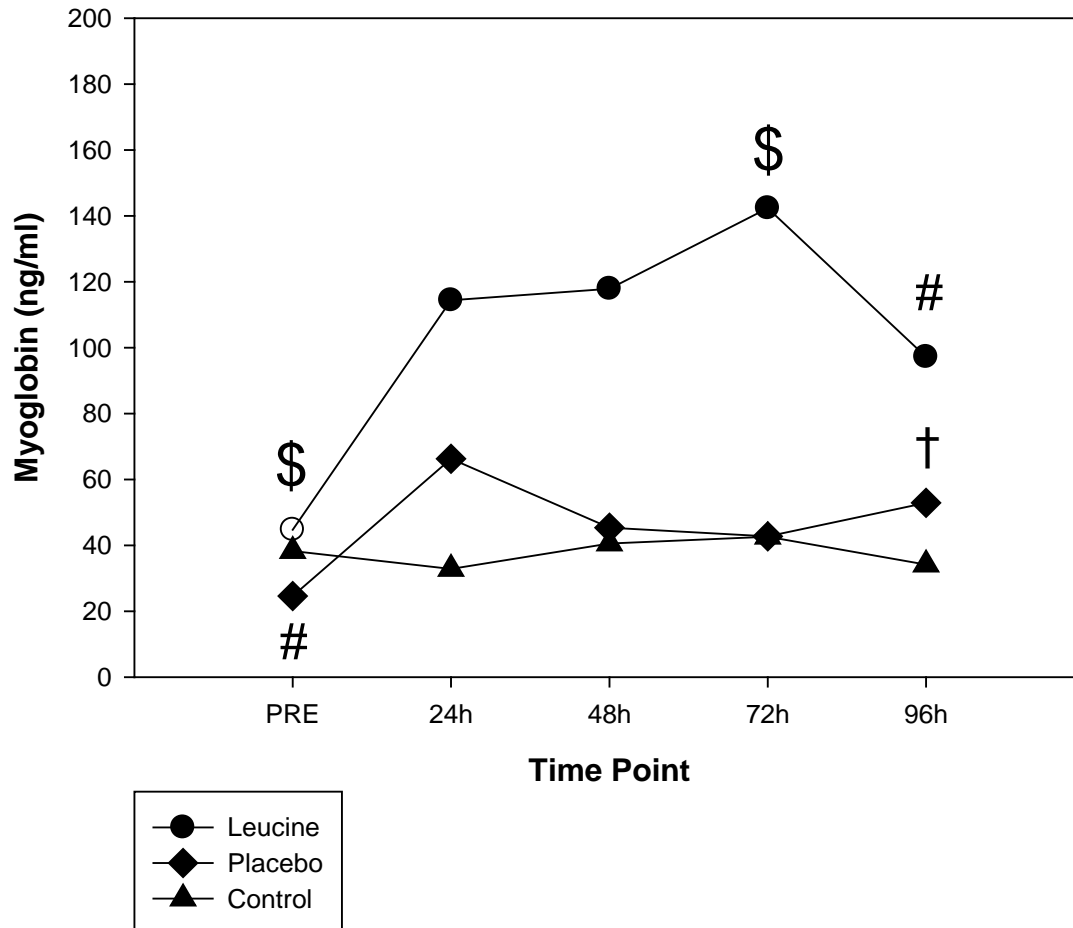


Figure 4. Myoglobin concentration activity following the resistance exercise protocol. \$ denotes significantly different from P group. # denotes significantly different from C group. † denotes significantly different from PRE.

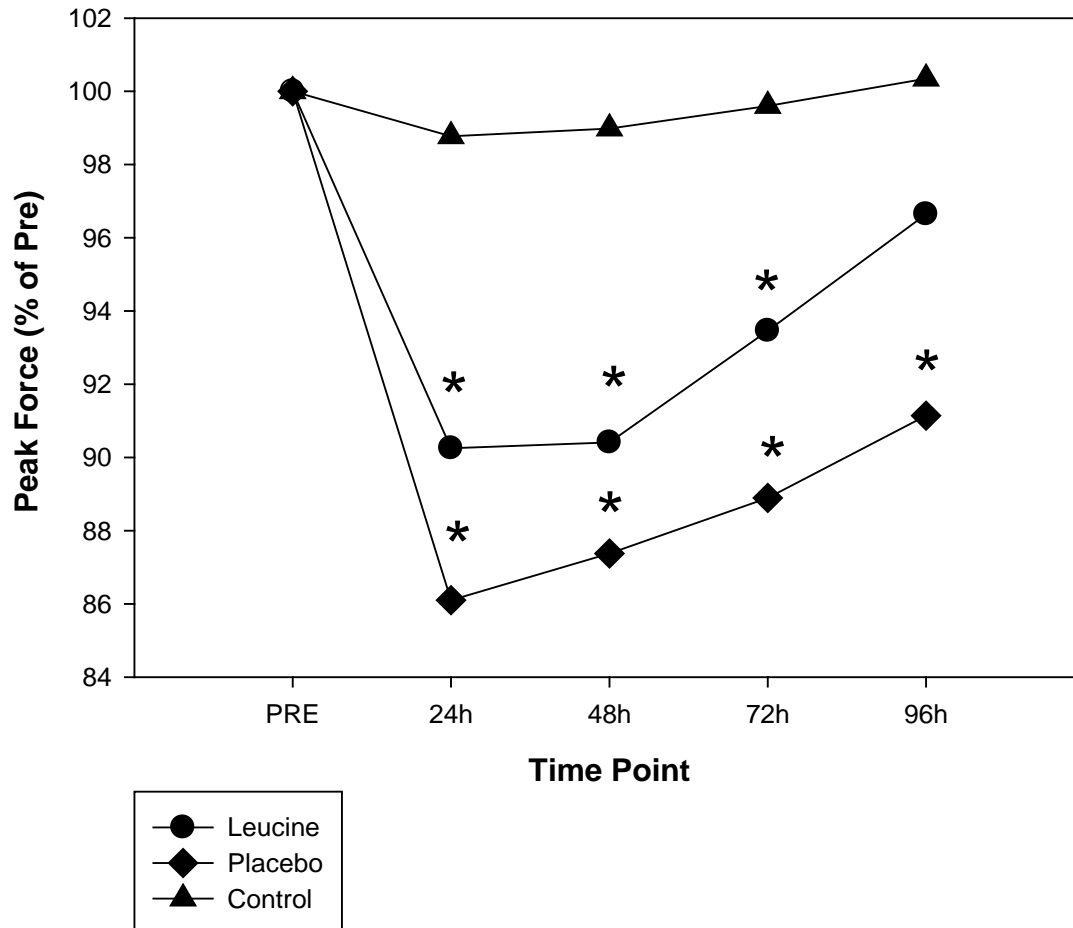


Figure 5. Peak force for an isometric squat following the resistance exercise protocol. * denotes significantly difference from PRE value and C group.

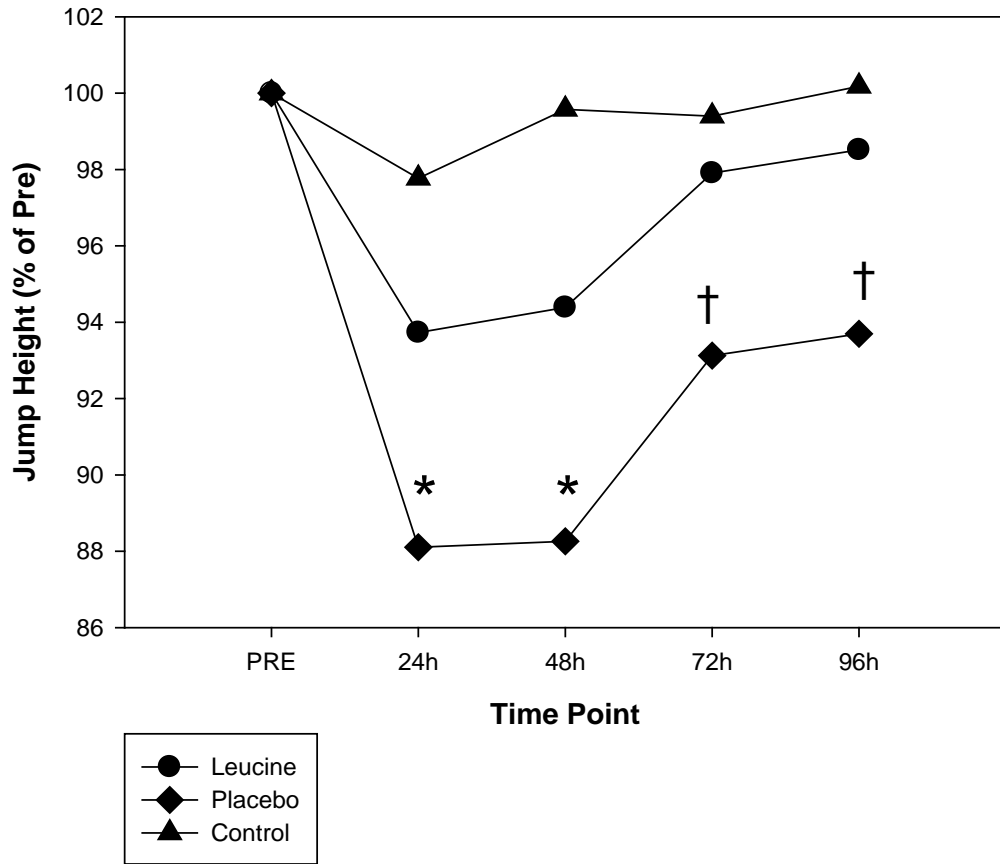


Figure 6. JH for SJ following the resistance exercise protocol. * denotes significantly difference from PRE value and C group. † denotes significantly different from PRE.

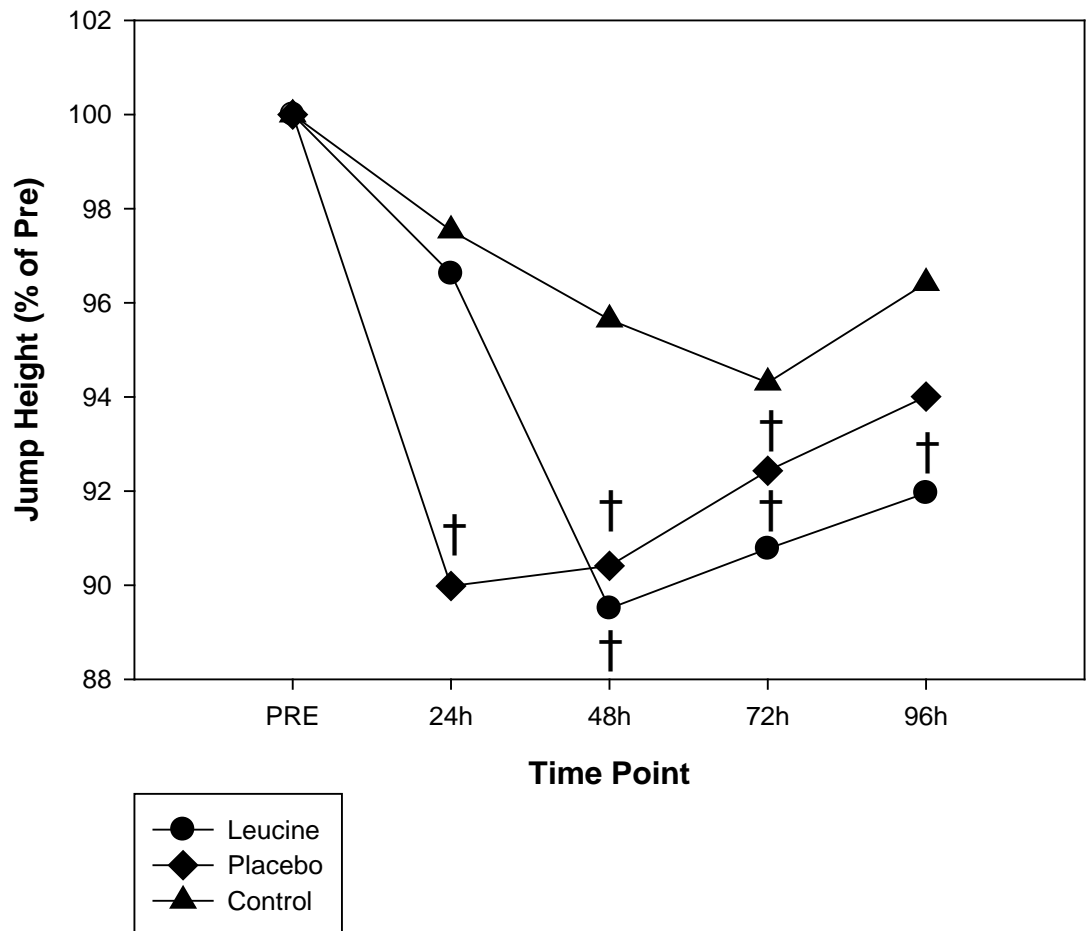


Figure 7. Maximum JH for CMJ following the resistance exercise protocol. † denotes significantly different from PRE.

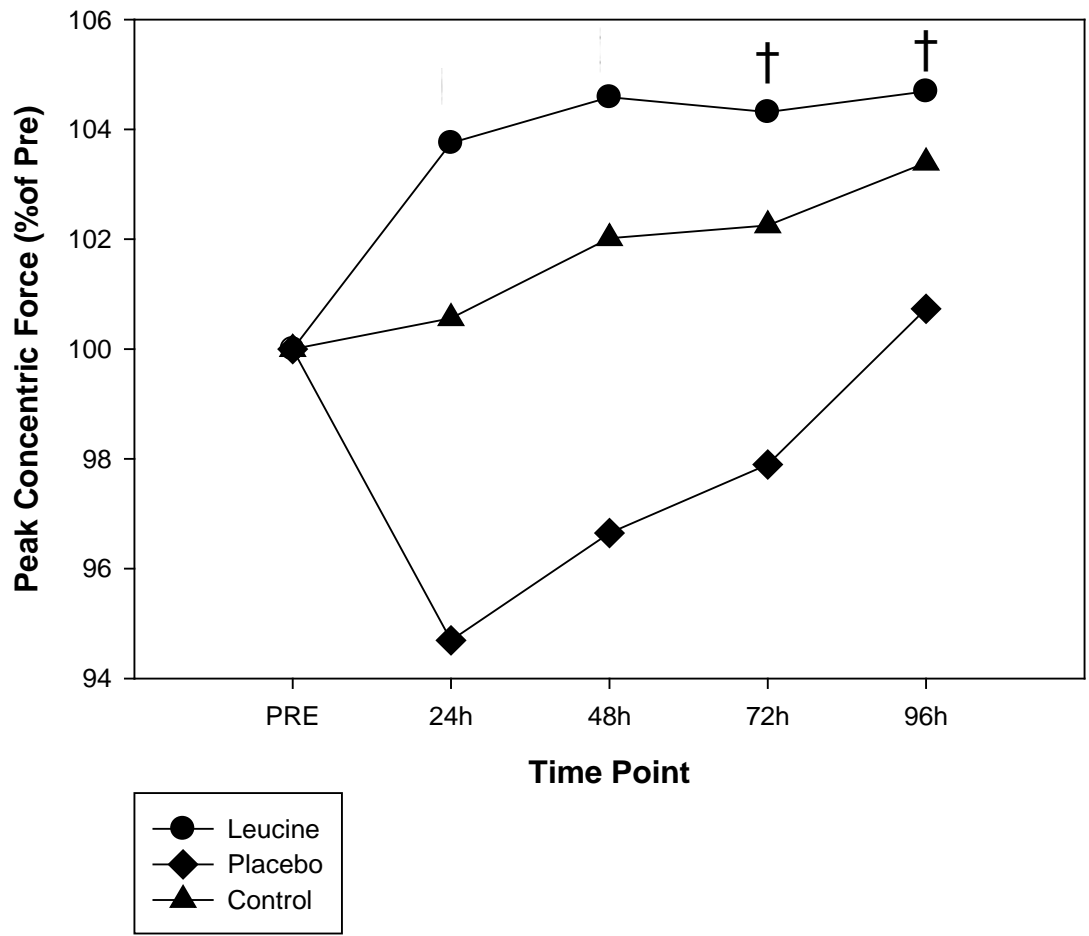


Figure 8. PCF for SJ following the resistance exercise protocol. † denotes significantly different from PRE. § denotes significantly different from P group. ‡ denotes significantly different from PRE and P group.

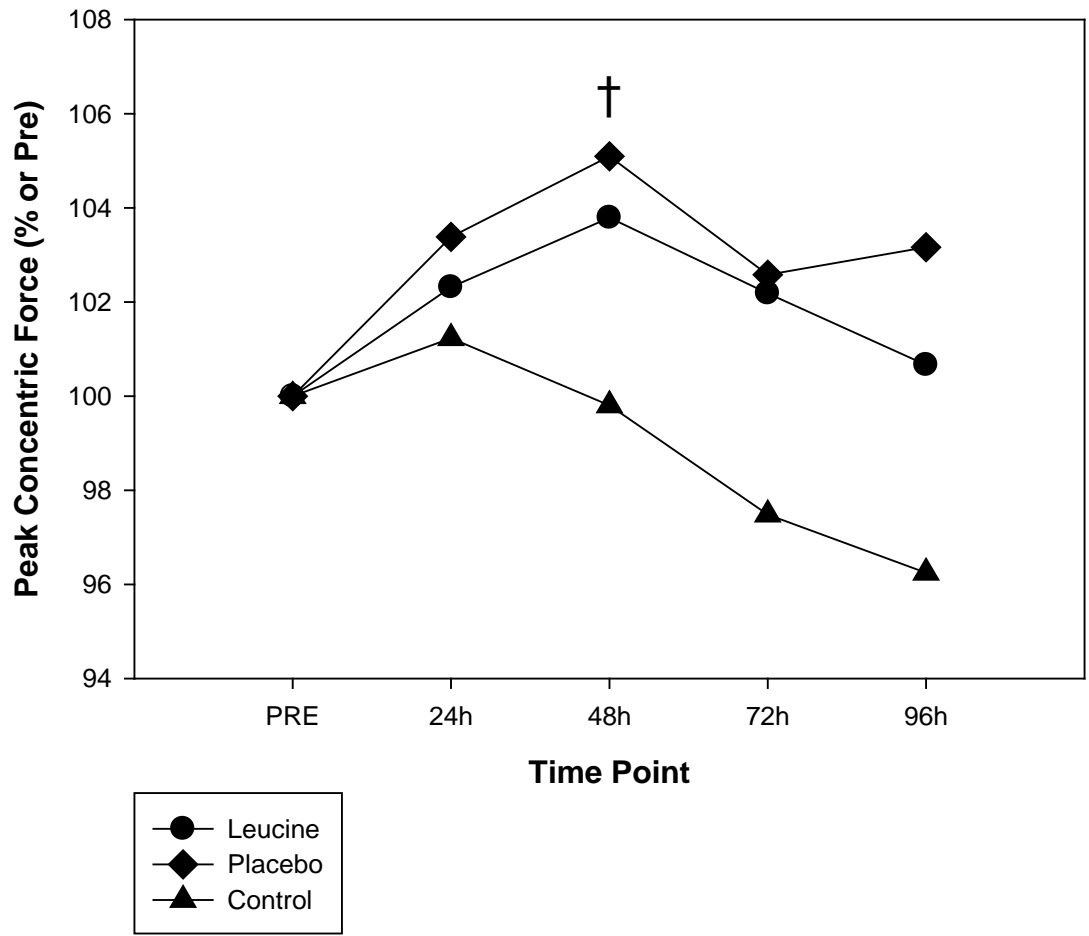


Figure 9. PCF for CMJ following the resistance exercise protocol. † denotes significantly different from PRE.

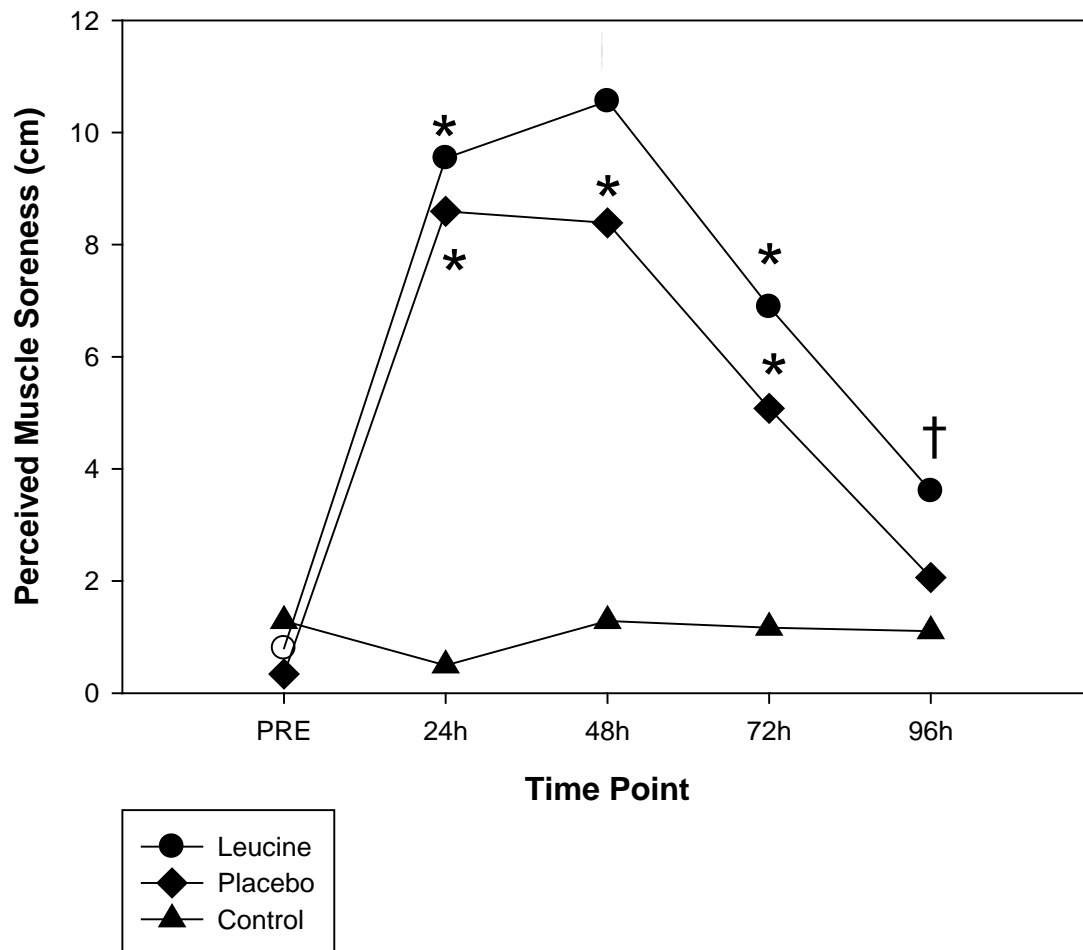


Figure 10. PMS following the resistance exercise protocol. * denotes significantly difference from PRE value and C group. † denotes significantly different from PRE. § denotes significantly different from P group.

APPENDIX A

Institutional Review Board Documentation

APPALACHIAN STATE UNIVERSITY

REQUEST FOR REVIEW OF HUMAN PARTICIPANTS RESEARCH

Please type and submit 1 copy to the Chairperson, IRB; c/o Graduate Studies and Research, John E. Thomas Building

- 1. Date** August 24, 2009
- 2. Project Title** Effects of Leucine Supplementation on Indices of Muscle Damage and Repair, and Muscle Function Following Resistance Exercise
- 3. Principal Investigator** Tyler J. Kirby
- 4. Telephone Number** (828) 406-4885
- 5. E-mail address** kirbytj@appstate.edu
- 6. Department/School** Health, Leisure, and Exercise Science, College of Fine and Applied Arts
- 7. Relationship to University** Student
- 8. Faculty Mentor** N. Travis Triplett
- 9. Mentor's E-mail** triplttnt@appstate.edu
- 10. This is a:** Thesis Project.
- 11. Funding source** National Strength and Conditioning Association
- 12. Projected data collection** September 15th, 2009 to April 15th, 2010
- 13. Have the investigators completed training in the use of humans in research?**
Yes

I have read Appalachian State University's Policy and Procedures on Human Subjects Research and agree to abide by them. I also agree to report any significant and relevant changes in procedures and instruments as they relate to participants to the Chairperson of the Institutional Review Board.

Principal Investigator

Date

CHECKLIST FOR RESEARCH INVOLVING HUMAN SUBJECTS

Please type and submit two copies to the Chairperson, IRB, c/o Office of Research and Graduate Studies. Respond to all questions. Attach additional sheets as needed. Staple all pages together when finished. Attach copies of questionnaires, non-standard tests, consent form, and other supporting documents.

1. Purpose of proposed research.

Previous investigations have shown that nutritional interventions, particularly protein ingestion, during intense training may decrease skeletal tissue breakdown. This may be of particular benefit to athletes that require increased recovery between training sessions or athletes participating in numerous competitive bouts within a short time frame. Investigations of isolated animal tissues have shown that leucine is the most important of the branched-chain amino acids needed to stimulate protein synthesis. Amino acid mixtures containing high levels of leucine have also been shown to attenuate exercise-induced muscle damage. Leucine also provides substrate for cholesterol synthesis, which may aid in recovery as exercise-induced muscle damage has been shown to decrease plasma cholesterol concentrations. Exercise-induced muscle damage may cause a decrement in muscle function which would influence force-generating capability. Decreases in force-generating capability may then limit subsequent muscular performance capability. However, no investigations in the human body have examined leucine alone and how it affects indices of muscle damage and cholesterol concentrations after intense resistance exercise. Therefore, the purpose of this investigation is to examine the effects of leucine supplementation on indices of muscle damage, plasma cholesterol concentrations and muscle function after a bout of resistance exercise.

2. Briefly describe your subject population. Will any individuals be excluded solely on the basis of gender, race, color, or any other demographic characteristic? If so, please explain.

Subjects will include 30 men experienced in resistance training and between the ages of 18 and 30. Subjects will be recruited primarily through announcements in the campus and local strength and conditioning facilities and will not be excluded solely on the basis of race, color, or any other demographic characteristic. Women will not be involved in the study in order to maintain a homogeneous subject population and to avoid hormonal influences of the menstrual cycle on exercise and recovery.

- 3. Give a brief description or outline of your research procedures as they relate to the use of human participants. This description should include, at least, the following: procedures; name and description of data gathering instrument(s) (attach copy if applicable); how the data will be collected (e.g. audio, video, written records); sample size; how long the procedure(s) will take; what, if any, relationship exists between the researcher(s) and participants.**

• Procedures

Participants in this experiment will visit the Neuromuscular Laboratory in the Holmes Convocation Center six times. Subjects will be asked to refrain from performing any type of resistance exercise or strenuous activity 48 hours prior to each of the first two testing sessions, as well as during the post-resistance exercise period (96 hours). All testing sessions will be preceded by a brief warm-up. The first testing session for all subjects will consist of collecting anthropometric measurements, as well as a one repetition maximum (1RM) in the leg press exercise. Subjects will also perform two trials for each of the following performance tests: isometric squat, static jump, countermovement jump and depth jumps from three heights (20 cm, 40 cm, 60 cm). The second session will involve a blood sample and the subjects performing the resistance exercise protocol (if they are in one of the two experimental groups) while ingesting one of the supplements based on their assigned group (supplement or placebo). The supplement will be mixed into water containing an artificial sweetener (Splenda) and the placebo beverage will consist of the artificially sweetened water without the supplement. Since the groups are independent (i.e. not a crossover design), the likelihood of the subjects discovering the true supplement beverage is low. The experiment will also be double-blinded. Doses will consist of 250 mg/kg of body weight for both leucine and placebo, with 7 doses being given over the five day testing period. Leucine supplementation is not toxic, even in extremely high doses (Baker, 2005) as well there are no known drug interactions. The RDA for leucine in adults is 45 mg/kg body weight, yet quantities of 360 mg/kg body weight per day for 6 months have been used with no adverse effects (Baker, 2005). The control group will only have the blood sample taken and will not perform the resistance exercise protocol nor have a supplement to ingest during this session. The resistance exercise protocol will consist of 5 sets of 20 maximal depth jumps from a height of 60 cm with a 10-second interval between jumps. A 2-minute rest will be given between sets. Subjects will then perform 6 sets of 10 eccentric contractions on the leg press with a weight equal to 120% of their concentric 1 RM. During all repetitions the eccentric portion of the movement will be performed at a 6-second tempo and a 2-minute rest will be given between sets. The third through sixth sessions (24 h, 48 h, 72 h, and 96 h post-resistance exercise) will consist of all of the subjects having a blood sample and then completing the initial performance tests (isometric squat, static jump, countermovement jump and depth jumps). The subjects' whole blood will be collected (after a 12-hour fast) through venipuncture of an antecubital vein, and analyzed at a later date for indices of muscle damage and plasma cholesterol. Perceived muscle soreness will be also assessed via a delayed-onset muscle soreness Likert scale at the second session and 24, 48, 72, and 96 hours post-resistance exercise time points. Dietary records will be kept for the 48-hours prior to testing as well as in the

recovery days following the testing. Dietary records will be analyzed for total caloric intake, as well for each macronutrient (carbohydrates, protein, and fat).

• **Name and description of data gathering instruments**

Force Plate (AMTI)

Linear Position Transducers (Celesco Transducer Products)

Custom programs designed using LabVIEW (Version 8.2, National Instruments)

Dietary analysis software (Food Processor™)

Biochemical Analysis Kits (Fisher Scientific and Sigma Chemical Co.)

• **How will the data be collected? (e.g., audio, video, written records)**

Data will be recorded during the testing sessions via a LabView program on the equipment which is interfaced to a laboratory computer. The subjects will fill out dietary records, and the delayed-onset muscle soreness scale. Blood results will be obtained through biochemical analysis and the values entered manually by the investigators in a spreadsheet.

• **Sample size**

30

• **How long will the procedures take?**

Two 1.5-hr sessions and four 30-min sessions

• **What, if any, relationship exists between the researcher(s) and the participants?**

None

• **What, if any, relationship exists between the researcher(s) and the agencies (e.g., schools, hospitals, homes)?**

None

4. Is deception involved?

Yes. Subjects in the experimental groups will be unaware of which supplement they are receiving during each testing session.

5. Do the data to be collected relate to illegal activities (e.g., drug use, abuse, assault)?

No

6. The benefits of this activity to the participants must outweigh the probable risks. To this end:

a. Describe the benefits to the individual participants and to society.

Subjects will be informed of their individual strength and power levels as well as resting and exercise-induced cholesterol concentrations and the comparative norms for their age. Subjects will also learn how their muscle tissue responds to strenuous exercise, as indicated by the markers of muscle damage. Subjects will be informed of how their participation advances the field of exercise science and how this applies to the athletic and general populations. Subjects will also be compensated for their participation in this study for the amount of no less than \$30.

b. Describe the potential risks to any individual participating in this project. Please explain any possible risks of psychological, legal, physical, or social harm. What provisions have been made to insure that appropriate facilities and professional attention necessary for the health and safety of the participants are available and will be utilized?

There are no inherent risks involved with this investigation except for the potential of muscle pulls or strains associated with the testing common to any type of physical activity. The risk of blood draws includes the possibility of local discomfort or minor bruising, and possible temporary lightheadedness upon rising. Care will be taken to avoid these side effects, and all blood samples will be taken by a trained phlebotomist. Chances of infection are minimal and all precautionary measures to minimize these occurrences will be taken. Testing will be monitored by qualified personnel (Certified Strength and Conditioning Specialist) with first aid and CPR certification. All procedures for the physical performances tests are previously published and are outlined in the standards for the field of Strength and Conditioning as supported by the National Strength and Conditioning Association.

7. Please describe how participants will be informed of their rights and how informed consent will be obtained and documented. Attach a copy of the consent form and any materials used in the recruitment of participants.

The study will be thoroughly explained to the subjects by the investigators and the subjects will be given the opportunity to demonstrate their clear understanding of their involvement in the investigation and to ask questions. A copy of the consent form will then be reviewed with each subject and once voluntary consent is given through a signature, the forms will be kept in a locked file cabinet in the Neuromuscular Laboratory.

8. The confidentiality of all participants must be maintained. To this end, please respond to the following.

a. How will the confidentiality of participants be maintained?

Subject identity will not be disclosed in any published documents or shared with anyone but the experimenters. All information collected will be kept confidential and disguised

so that no personal identification can be made and all experimental data will be identified by number only. Confidentiality of all subjects will be maintained by keeping subject files under lock and key. Individual data will not be reported in results of final publication.

b. How will confidentiality of data be maintained?

All data analysis and results will be conducted/maintained using identification numbers and all subject data files will be locked in the Neuromuscular Laboratory until all final dissemination of the data, after which subject files will be destroyed.

c. Describe the process of final disposition of the data. How long will the data be stored and how will they be destroyed?

Data will be analyzed and be presented and reported in manuscript format and submitted to a peer-reviewed journal for publication. Papers will be shredded and data files deleted after 5 years.

d. How are participants protected from the future harmful use of the data collected in this protocol?

Study result information will be kept confidential and disguised so that no personal identification can be made. Subjects will be protected by avoiding the use of personal names and/or photographs (unless consent is given) and also through the use of identification numbers.

I have read Appalachian State University's Policy and Procedures on Human Subjects Research and agree to abide by them. I also agree to report any significant and relevant changes in procedures and instruments as they relate to participants to the Chairperson of the Institutional Review Board.

Signature of Principal Investigator

Date

APPALACHIAN STATE UNIVERSITY

Informed Consent for Participants in Research Projects Involving Human Subjects

Title of Project: Effects of Leucine Supplementation on Indices of Muscle Damage and Recovery, and Muscle Function Following Resistance Exercise

Investigator(s): Tyler Kirby and N. Travis Triplett

I. PURPOSE

Previous investigations have shown that changes to the different nutrients consumed in the diet, particularly the type of protein, during intense training may decrease skeletal muscle breakdown. This may be of particular benefit to athletes that require increased recovery between training sessions or athletes participating in numerous competitive bouts within a short time frame. Investigations in animals have shown that leucine is the most important of a special group of amino acids, known as the branched-chain amino acids, needed to stimulate protein synthesis. Amino acid mixtures containing high levels of leucine have also been shown to reduce exercise-induced muscle damage which can result in decreased physical performance. Leucine may also have a role, along with cholesterol, in recovery from intense exercise. However, no investigations in the human body have examined leucine alone and how it affects muscle tissue changes and hormone levels after intense resistance exercise. Thus, the purpose of this investigation is to examine the effects of leucine on indicators of muscle damage as well as on blood cholesterol levels, which is required for skeletal tissue repair after a bout of resistance exercise.

II. PROCEDURES

As a participant in this experiment you will be asked to visit the Neuromuscular Laboratory on 6 occasions for periods ranging from 30 minutes up to 1.5 hours over the course of approximately 2 weeks. You will be required to engage in physical activities such as a maximal leg press, a maximal isometric squat, and maximal depth jumps. If selected to either experimental group you may be asked to ingest a nutritional supplement or placebo during these physical activities. The nutritional supplement will consist of 250 mg/kg body weight of leucine, an essential amino acid, mixed in water with 3g of artificial sweetener (Splenda). The placebo mixture will contain just the 3g artificial sweetener in water. You have an equal and random chance of being selected to the control group or one of the two experimental groups. You will have a small blood sample (10 ml) taken from your arm at the beginning of the second exercise session as well as 24, 48, 72, and 96 hours after the second exercise session. Blood draws will be performed by a trained phlebotomist. Prior to the second exercise session you will be asked to fast for 12 hours and to refrain from strenuous exercise for 48 hours. You will also be asked to fast for 12 hours for the 24, 48, 72, and 96 hour-testing sessions, as well

as to perform no strenuous exercise in these recovery days. You will also be instructed how to keep a food diary for the 48 hours prior to and in the days following the second exercise session.

III. RISKS

There are no inherent risks involved with this investigation. The nutritional supplement will consist of 250 mg/kg body weight of leucine, an essential amino acid, mixed in water with 3g of artificial sweetener (Splenda™). The placebo mixture will contain just the 3g artificial sweetener in water. Leucine is found in the diet in nearly every protein source (meat, fish, dairy, eggs, etc.), and Splenda™ is the newer, more commonly used sweetener in many low-sugar foods, so you have consumed both of these substances on a regular basis. The amount of leucine in the supplemental protocol is higher than what is consumed in a typical daily diet, but several research studies have used greater than this amount and have shown no side effects. There is a very slight possibility of some nausea and/or gastrointestinal distress whenever consuming any supplement in high dose.

With any form of exercise, there is the possibility of muscle pulls or strains associated with the physical activity. To prevent these, adequate warm-up procedures will be followed and testing will be monitored by qualified personnel with appropriate certifications to minimize these risks. Standardized procedures for these types of physical activity testing will be followed according to the professional guidelines of the National Strength and Conditioning Association and previously published investigations.

The risks of infection, thrombosis, or embolism apply during blood draws although every precaution has been made to minimize these risks. Universal precautions will be used throughout all blood collections, which will be performed by a trained phlebotomist. The researchers in this investigation assume all blood or body tissues are potentially infectious. In the case of exposure of an experimenter by your blood or tissue, we will analyze that blood for HIV and hepatitis. If your blood or tissue has to be analyzed for HIV and hepatitis, you will be notified of the result.

IV. BENEFITS

In addition to the monetary payment, you will be given results identifying your maximum strength and force produced in comparison to norms for your age group. In addition, your resting and exercise-induced cholesterol and muscle enzyme concentrations will be determined in comparison to norms for your age group.

V. EXTENT OF ANONYMITY AND CONFIDENTIALITY

Anonymity of subjects will be maintained at all times during and after your involvement in this study by the assignment of a number by which only the investigators can identify your data. Individual data collected will remain confidential and will not be disclosed in any published document or shared with anyone but the experimenters.

VI. COMPENSATION

Funds in the amount of \$30.00 have been set aside for each subject for participating in the investigation. You understand that you may choose to discontinue participation in the study at any time and will not be held to completion of the study against your will. However, you have been told that compensation (\$30) is contingent upon full completion of the study. No additional funds for any injury or illness resulting from participation in this study have been allotted. You have been told that in the event of physical injury resulting from the research procedures, immediate first-aid is provided free of charge. Appalachian State University assumes no commitment to provide monetary compensation or free medical care to you in the event of a study-related injury.

VII. FREEDOM TO WITHDRAW

You are free to withdraw from the study at any time without penalty. If you choose to withdraw, you will be compensated for the portion of the study which you completed. Subjects are also free not to answer any questions or respond to experimental situations that they choose without penalty. There may be circumstances under which the investigator may determine that a subject should not continue the investigation. In that case, the subject will be compensated for the portion of the project completed. You must be 18 years of age to participate in this study. If you feel your rights have been violated or you have questions, contact the IRB Administrator, Julie Taubman, at 828-262-7981.

VIII. APPROVAL OF RESEARCH

This research project has been approved, as required, by the Institutional Review Board of Appalachian State University.

IRB Approval Date

Approval Expiration Date

IX. SUBJECT'S RESPONSIBILITIES

I voluntarily agree to participate in this study. I have the following responsibilities:

- No participation in a structured lower-body resistance exercise program in the six months prior to the investigation. No use of ergogenic aids in the previous three months. No prescription drugs that impair cholesterol synthesis or reduce inflammation.
- Arrive at the testing site in a fasted condition, without having engaged in strenuous activity for 48 hours prior to my scheduled test time, and having recorded my food intake for the previous 48 hours.

- Complete a single 1 hour testing session involving maximum lifting and physical performance testing, and some basic body size measurements.
- Complete a single 1.5 hour testing session involving a blood sample, perceived-soreness evaluation, resistance exercise, and jumping, and maybe ingesting a nutritional supplement.
- Come to the laboratory four additional times for only a blood sample, perceived-soreness evaluation, and physical performance testing.

X. SUBJECT’S PERMISSION

I have read and understand the Informed Consent and conditions of this project. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent:

_____ Date _____
 Subject signature

_____ Date _____
 Witness (Optional except for certain classes of subjects)

Should I have any questions about this research or its conduct, I may contact:

<u>Tyler Kirby</u>	<u>828-406-4885</u>	<u>kirbytj@appstate.edu</u>
Investigator	Telephone	e-mail

<u>Dr. N. Travis Triplett</u>	<u>828-262-7148</u>	<u>triplttnt@appstate.edu</u>
Faculty Advisor	Telephone	e-mail

<u>Dr. Tim Ludwig</u>	<u>828-262-2712</u>	<u>irb@appstate.edu</u>
Chair, IRB	Telephone	e-mail

VITA

Tyler John Kirby was born in Kitchener, Ontario on December 17th 1983. After graduating from Sandwich Secondary School, he attended the University of Windsor for two years. After his sophomore year, he transferred to St. Francis Xavier University on a football scholarship where he graduated with a Bachelor of Science in Human Kinetics with honors. In fall of 2008, he enrolled at Appalachian State University and was awarded a Master of Science in Exercise Science in May 2010. Tyler will be attending the University of Oklahoma in the fall of 2010 to pursue his Doctorate of Philosophy in Exercise Physiology. Following completion of his Ph.D. he plans on pursuing post-doctorate positions or teaching and conducting research at an academic institution. Tyler's parents are Dan and Laurie Kirby who reside at 48 Hope and Carton, Christiansted, St. Croix in the United States Virgin Islands.