ABSTRACT

The Effects of Eight Weeks of Heavy Resistance Training and Branched Chain Amino Acid and Carbohydrate Supplementation on Muscle Performance and Body Composition

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Branched chain amino acid supplements are becoming one of the more popular and well known supplements currently on the market. It is thought that branched chain amino acids support the body during resistance training by supplying the muscles with an excess supply of branched chain amino acids in order to quickly repair muscle, supplying the muscles with energy, and increasing muscle protein synthesis. This study evaluated how eight weeks of supplementation with branched chain amino acids and carbohydrates affected body composition and muscle performance in untrained males between the ages of 18 and 35. In other short term studies there are significant improvements that can be made with supplementation (Kerksick, et al., 2006). This study evaluated the long term effects that may be seen with supplementation.

The Effects of Ten Weeks of Heavy Resistance Training and Branched Chain Amino Acid Supplementation on Muscle Performance and Body Composition

by

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A Thesis

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CHAPTER ONE

Introduction

Branched-chain amino acids (BCAA) make up about one third of skeletal muscle tissue within the human body and consist of leucine, isoleucine, and valine (Mero, 1999). Not only are BCAAs a key component of muscle tissue, there seems to be some correlation between BCAAs and increases in muscle protein synthesis (MPS) (Shimomura, Murakami, Nakai, Nagasaki, & Harris, 2004). Luecine is thought to possess the greatest capability of increasing MPS. After six weeks of leucine supplementation, there was improvement in rate of percieved exertion, muscular endurance, muscle power and muscular work (Crowe, Weatherson, & Bowden, 2006).

BCAA supplementation has become a popular nutritional adjunct to resistance training because it is thought to augment increases in muscle mass, strength, and performance beyond what is normally observed with training alone. The available data involving prolonged BCAA supplementation (weeks or months) is severely lacking. In a study using rats, BCAA supplementation was observed to increase the time to exhaustion and endurance performance (Calders, Pannier, Matthys, & Lacroix, 1997).

In regard to the mechanism(s) in which BCAAs affect MPS, BCAAs has been shown to increase activity of the Akt/mTOR pathway, specifically the levels of mammalian target of rapamycin (mTOR), 70-kDa ribosomal protein S6 kinase (p70S6K), and eukaryotic initiation factor 4E-binding protein 1(eIF4E-BP1) (Blomstrand, Eliasson, Karlsson, & Kohnke, 2006), (Liu Z. , Jahn, Long, Fryburg, Wei, & Barrett, 2001). Both

p70S6K and eIF4E-BP1 have been identified as key components to the Akt/mTOR signaling pathway (Liu Z. , Jahn, Long, Fryburg, Wei, & Barrett, 2001). Upon Akt/mTOR pathway activation, MPS is enhanced, thereby creating a net anabolic effect. The enhancement of MPS is likely related to the increases in mTOR, p70S6K, and eIF4E-BP1 which are involved in translation of muscle-specific mRNA (Blomstrand, Eliasson, Karlsson, & Kohnke, 2006).

During exercise, BCAA oxidation is increased based on exercise intensity (Matsumoto, Koba, Hamada, & Mitsuzono, 2009). When participating in high intensity exercises, the body readily utilizes free-flowing BCAAs by oxidation instead of catabolizing muscle protein for an energy source. Therefore, BCAAs seem to play an inhibitory role in protein degradation (Tom & Nair, 2006). The inhibitory properties of BCAAs have been demonstrated when rats are put into catabolic conditions through starvation. While in this catabolic condition, BCAAs were shown to increase MPS and minimize protein degradation (Hong & Layman, 1984). In addition to promoting MPS, BCAAs may help to minimize muscle protein degradation during high-intensity exercise such as heavy resistance training. Even though the specific mechanism in which BCAAs are able to regulate MPS while concomitantly minimizing protein degradation is not completely known and still remains to be determined (Nair & Short, 2005), it is plausible to consider that supplementing with BCAAs during resistance training may provide a nutritional platform for enhancing fat-free mass and body composition (Kerksick, et al., 2006).

Amino acid supplementation combined with carbohydrates has been shown to have an even greater effect on MPS (Rasmussen, Tipton, Miller, Wolf, & Wolfe, 2000).

It is possible that by combining amino acids and carbohydrates there is an even greater response in skeletal muscle. When essential amino acids are combined with carbohydrates and taken for a 28 day period, the supplements seemed to help maintain muscle mass in participants who were under bedrest. The supplemented participants' muscle mass was saved because the body switched into a more anabolic state versus the unsupplemented control group who lost muscle mass (Waknine, 2004).

Improvements in MPS with BCAA supplementation have primarily been shown to occur in short term studies. However, individuals who are typically taking BCAA supplements are using them for long-term improvements. For long term improvements there is little data to substantiate whether or not BCAA supplementation when combined with resistance training will effectively increase muscle mass and strength. Because of this paucity of data, the question that has yet to be answered is if BCAA supplementation will provide an ergogenic adjunct to resistance training.

Purpose of Study

The purpose of this study was to compare the effects of 8 weeks of heavy resistance training with carbohydrate and/or BCAA supplementation on body composition (fat-free mass, fat mass, percent fat) and muscle performance (strength, power, endurance).

Hypotheses

H_1: Eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly increase upper body muscle strength when compared to CHO.

H_2: Eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly increase lower-body muscle strength when compared to CHO.

H_3: Eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly increase upper-body muscle endurance when compared to CHO.

H_4: Eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly increase lower-body muscle endurance when compared to CHO.

H_5: Eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly increase lower-body muscle power when compared to CHO.

H_6: Eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly increase fat-free mass when compared to CHO.

H_7: Eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly decrease fat mass when compared to CHO.

Delimitations

The study was delimited in the following manner:

1. 30 recreationally active males between the age of 18-28 years of age participated in the study
2. Participants were recruited from Baylor University student population and were recruited through flyers and posters around the campus.
3. Participants were randomly assigned to either a BCAA or placebo group
4. Participants performed 4 days a week of lower body resistance training at 70-80% of the 1-RM.
5. Participants ingested their BCAA/placebo supplement 30 minutes prior to training and immediately afterwards.

6. The participants in this study did not change their current nutritional intake or their current workout routine
7. All testing was performed in the Exercise and Biochemical Nutrition Lab and Exercise and Sport Nutrition Lab at Baylor University in the Marrs-McLean Gym according to all policies and procedures within each respective laboratory.

Limitations

The study was limited in the following manner:

1. The sample size was limited to only those volunteers who came forward to participate.
2. The sensitivity of the equipment.
3. Honesty and effort of the participants in familiarization and testing sessions.
4. The participants did not make changes to their dietary intake.

Assumptions

The following assumptions were involved with the study:

1. Participants were apparently healthy and met all guidelines set for the study.
2. Participants were recreationally active but are not resistance trained.
3. Participants followed instructions that were set out during familiarization phase.
4. All equipment was in working order and is reliable.
5. All methods were current, accurate, and reliable.

CHAPTER TWO

Review of literature

Branched-chain amino acids play multiple roles in the body that may support muscle performance during resistance exercise. The use of BCAA supplements are starting to become more popular for these reasons. There seems to be some amount of BCAAs used as an energy source in the body. The BCAAs play a regulatory role in MPS that may increase fat-free mass and stimulate improvements in muscular strength. The increase in MPS is speculated to produce a net anabolic effect and help reduce muscle tissue damage, thereby allowing for an increased performance (Sharp & Pearson, 2010). The increase in muscle protein synthesis due to BCAA supplementation may lead to increases in muscular strength but the data are limited.

BCAAs as an Energy Source

In the body, BCAAs are 3 out of the 9 essential amino acids and comprise one-third of all the amino acids found within skeletal muscle (Shimomura, Murakami, Nakai, Nagasaki, & Harris, 2004). It has been speculated that these amino acids play an important role in skeletal muscle while in a catabolic state, for example, during exercise. During exercise, carbohydrates and fat are oxidized in large amounts (10-20 fold) allowing for greater utilization for energy. BCAAs are thought to provide a significant amount of energy during exercise but it seems to only provide a 2-3 fold increase in oxidation during exercise (Gleeson, 2005). While this may not be a large increase in oxidation during exercise it is showing that BCAAs do provide some energy for muscles.

By using BCAAs as an energy source the skeletal muscle is able to spare its own proteins when performing.

In some studies with BCAAs, it has been speculated that leucine may be used by skeletal muscle as a fuel source under some conditions. In a 20-minute bout of aerobic exercise at 70% VO2 max, it has been observed that when glycogen stores are reduced that skeletal muscle may actually use leucine as a fuel source for muscle contraction, but not when there are normal levels of glycogen stores. With leucine supplementation, or 30-35% leucine in a mixture of BCAA solution, there may be decreased muscle protein degradation as well as glycogen sparring, which can reduce the needs on muscle glycogen stores (Mero, 1999). If the body is able to use BCAA as a fuel source, it can help prevent muscle damage from exercise and allow for more MPS to occur.

During endurance exercise, it is presumed that the body can utilize BCAAs as a fuel source for work which can allow for more MPS, thereby creating more fat-free mass. A single ingestion of two grams of BCAAs results in an increase of plasma BCAA concentration and skeletal muscle uptake during moderate-intensity exercise. When supplementing with BCAAs for a one week regiment, it has been noted that it seems to affect one of the key regulatory factors for glucose metabolism and suppresses glycogen consumption in skeletal muscle (Matsumoto, Koba, Hamada, & Mitsuzono, 2009). The decrease in the glycogen consumption in skeletal muscle during exercise could be correlated to the leucine being used by the muscle as an energy source. By using leucine, the body is able to spare the muscle and hepatic glycogen for later consumption when needed.

Unlike the other amino acids, BCAAs are primarily oxidized in the skeletal muscle. This effect seems to be enhanced during exercise more than at rest. An oral supplementation of 77 mg/kg bodyweight before exercise seems to increase intracellular and arterial BCAA levels during exercise, which suppresses muscle catabolism during exercise (Shimomura, et al., 2006). The increase in the intracellular levels of BCAAs could be a result of the increase of intake allowing for oxidation. When this occurs, the BCAAs that are absorbed by the muscle are then easily accessible by the muscle as a fuel source instead of breaking down current muscle tissue or trying to utilize the glycogen stores.

BCAAs and Muscle Protein Synthesis

BCAAs are a possible source of energy for muscle during catabolic states but when it comes to MPS there seems to be more studies that promote this possibility because of its implications for a large population. During exercise the branched-chain α-keto acid dehydrogenase (BCKDH) complex is activated and oxidizes BCAAs during exercise training. By utilizing the BCAAs during exercise, the body is able to diminish the amount of muscle soreness following exercise and it is presumed that protein synthesis is stimulated while at the same time protein breakdown is being suppressed (Shimomura, Murakami, Nakai, Nagasaki, & Harris, 2004). When BCAAs are oxidized during exercise, it can activate mTOR and a sequential activation of p70S6K and eIF4E-BP1. This is important because when there is exercise without BCAAs or without adequate amounts of BCAAs, there is only a partial activation of p70S6k, thereby limiting the amount of possible MPS (Blomstrand, Eliasson, Karlsson, & Kohnke, 2006).

Without the complete activation of p70S6k, the amount of MPS is likely decreased. This highlights the importance of BCAAs in skeletal muscle during exercise.

These same findings can be seen when experimenting with rats which are put into catabolic states. After being put into either a 24-hour, 72-hour or 5-day starvation state by eating a protein-free diet, the soleus and extensor digitorum were extracted and added into a leucine enriched media. This study was able to show that at least under these specific catabolic conditions that leucine was able to stimulate muscle protein synthesis (Hong & Layman, 1984). Even under extreme catabolic conditions, the ability to use the leucine and stimulate protein synthesis allows for an idea of what happens when participating in high intensity resistance training that puts the body in catabolic states.

With supplementation, BCAAs are known to induce anabolic effects in human muscle tissue by increasing the amount of MPS. It seems to be that this occurs because of the activation of mammalian target of rapamycin (mTOR), which also stimulates the 70-kDa ribosomal protein S6 kinase (p70S6k) (Yang, Chi, Burkhardt, Guan, & Wolf, 2010). It is seen that increases in MPS after ingestion of BCAAs is due to the elevated levels of mTOR, p70S6k, and eukaryotic initiation factor 4E-binding protein 1 (eIF4E-BP1) without the increase in insulin levels (Rennie, Bohe, Smith, Wackerhage, & Grennhaff, 2006). When there is an increased amount of BCAAs in the body, there seems to be a cascading effect which activates a large number of other pathways that collectively increase the amount of MPS, and this effect seems to be even more increased with resistance training.

In an experiment using male Wistar rats, either 9 or 21 months old, they went through a one-month acclimation period to become accustomed to surroundings and food

so that stress from the environment change would not skew the results. They were then tested on a 10-day experimental diet with half of each group receiving either the leucine-enriched or alanine meal. On the last day, half of each group was not fed so that they would be in a post-absorptive state. The rats were then euthanized and the gastrocnemius and soleus muscles were taken to measure protein synthesis. The rats that were given the leucine-enriched meal had an increase in the phosphorylation state of p70S6K in both groups (Rieu, et al., 2003). The increase in p70S6K is known to cause an increase in protein synthesis in human skeletal muscle.

Another protein that has been studied along with the p70S6K is the eIF4E-BP1. Both of these proteins are known for their ability to regulate the signal transduction pathways for protein synthesis. In the study there were 7 volunteers (5 men and 2 women) who were each studied twice in random order. For the first 3 days subjects ate eucaloric flesh-free diets from the University of Virginia General Clinical Research Center metabolic kitchen, and then admitted themselves the night before experimentation. After eight hours of fasting, the subjects were given an intravenous solution of BCAAs. Blood samples were taken 30 minutes prior to the end of the basal period at 10 minute intervals, the last 30 minutes of 3 hours at 10 minute intervals, and 30 minutes of the last 6 hours at 10 minute intervals. Muscle biopsies were also taken from the vastus lateralis muscle and frozen to analyze the eIF4E-BP1 and p70S6K. It was determined that there were significant amounts of these two proteins that help promote significant anabolic signaling increasing MPS (Liu Z. , Jahn, Long, Frybug, Wei, & Barrett, 2001). These two proteins both show to play a significant role in MPS in human skeletal muscle and

they seem to be activated by the use and activation of BCAAs and both seem to work on the same or similar pathways as mTOR.

In a study to see if mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK1/2) and p70S6K were affected by the ingestion of a BCAA supplement, seven male subjects were used for a double blind crossover study. The subjects performed 4 sets of 10 repetitions with 5 min rest and used cycle ergometer at 100W for 10 minutes. Before warm-up, 15 minutes into the exercise, and at 15, 30, 60, and 90 minutes after the subjects ingested either a mixture of BCAA (45% leucine, 30% valine, 25% Isoleucine) or flavored water. It was seen that there was a 3.5 fold increase in P70S6K in the BCAA subjects and there were also significant increases in ERK1/2 and p38 MAPK (Karlsson, Nilsson, Nilsson, Chibalin, Zierath, & Blomstrand, 2004). The increases seen in this study are all known pathways for increasing muscle protein synthesis in the body. It is seen that BCAA supplementation with resistance exercise can cause significant increases leading to MPS.

The exact roles that BCAAs play in MPS and protein regulation is still yet to be determined; however, what we do know about BCAAs is that they do seem to regulate gene transcription and translation by activating proteins such as mTOR. This activation of mTOR leads to the activation of p70S6K and eIF4E-BP1 by initiating the translation of new muscle proteins (Nair & Short, 2005). If there is not a significant source of BCAAs in the body during exercise, then the body can enter into a catabolic state and will begin to break down the muscle proteins to use for energy stores. This also leads to a decrease in the activation of p70S6K and eIF4E-BP1 which will cause less MPS to occur.

Carbohydrates and Muscle Protein Synthesis

Carbohydrates by themselves seem to play a role in muscle protein synthesis in the body. It has been clearly noted that carbohydrates seem to play a role in increasing mTOR activation and it seem that carbohydrate ingestion seems to increase MPS and inhibit muscle protein breakdown (Drummond, Dreyer, Fry, Glynn, & Rasmussen, 2009). The Akt/mTOR pathway is one of the major pathways that can cause muscle protein synthesis to occur. It seems that when carbohydrates are ingested that the body is able to turn over the carbohydrates so that they can be used as energy and activate the Akt/mTOR pathway which can promote the uptake of amino acids, glucose, iron, and lipoproteins into the muscle cells, thereby aiding in muscle protein synthesis (Feng, 2010). While there seems to be an increased activation of mTOR, it is thought that the main reason the body becomes more anabolic after ingestion of carbohydrates is the fact that it shows to progressively decrease the amount of muscle protein breakdown (Borsheim, Cree, Tipton, Elliot, Aarsland, & Wolfe, 2004). This decrease in muscle protein breakdown could be due to having the energy source readily available to be used or it is thought that when there is an increase amount of carbohydrates ingested that it inhibits the AMPk processes which break down muscle protein for energy use (Wilkinson, et al., 2008). By decreasing the amount of muscle protein breakdown and increasing the activation of the Akt/mTOR pathways the skeletal muscles become more anabolic and are able to start producing more muscle proteins to increase muscle mass.

BCAAs, Carbohydrates, and Muscle Protein Synthesis

When combined with carbohydrates, BCAAs seem to induce an increased effect. We know already that by ingesting carbohydrates before, during and after exercise the

body is able to utilize these stores for energy rather than breaking down muscle protein. It has been seen that by combining essential amino acids and carbohydrates muscle anabolism is enhanced to a greater extent than when each is used separately. The combined effect between carbohydrates and essential amino acids seems to attenuate maximize anabolic responses by attenuating protein degradation (Bird, Tarpenning, & Marino, 2006). This increase in muscle anabolism is due to an increase in muscle protein synthesis. It is possible that the combined effect of the two could greatly increase the muscular gains from resistance training. In another study researchers had 32 subjects fast for 4 hours and then they performed a single bout of resistance training and during the subjects ingested carbohydrate (CHO) mixture, essential amino acid (EAA) mixture or a EAA+CHO mixture. After the resistance training bout, it was seen that the combination of the carbohydrates with essential amino acids suppressed exercise induced cortisol levels and through the altered balance in the body's catabolic state there is a favoring effect towards the conservation of myofibrillar protein levels (Bird, Tarpenning, & Marino, 2006). This decrease in catabolic hormones in the body and the conservation of myfibrillar protein helps lead to a greater increase in muscle protein synthesis. One thing that seems to aid this process is that after ingesting EAA+CHO there is an increased stimulation of mTOR. It is thought that this enhanced activation of mTOR signaling pathway is playing a large role in the increase in muscle protein synthesis after ingesting EAA+CHO with resistance exercise (Dreyer, et al., 2008). This seems to be the trend that, with the combination of EAA+CHO, there is an increase in muscle protein sythesis in muscle fibers. But in one study they found that the ingestion of EAA+CHO did not

show any benefits to muscle protein synthesis compared to the individuals who execised while fasted (Fujita, Dreyer, Drummond, Glynn, Volpi, & Rasmussen, 2009).

BCAA on Strength

BCAAs have been shown to improve grip strength after 30 days of supplementation. One study using 10 male subjects had them ingest orally a 14 g/day (50% L-Leucine, 25% L-Isoleucine, 25% L-Valine) supplement for 30 days. Before the supplementation began the participants went through baseline testing and did testing at the end of the 30 day period. At the end of the 30-day supplementation period, there were improvements seen in increases in fat-free mass, grip strength, and VO2 max (Candeloro, Bertini, Melchiorri, & De Lorenzo, 1995). The improvement in grip strength was seen without extra resistance training of the muscle. This leads to the assumption that it was the increase in BCAAs in the body allowing for these improvements to be noted. By having the increase in BCAAs the body was able to increase MPS, therefore, increasing muscular strength. Over a 10-week study with heavy resistance training and studying different types of proteins as well as a whey protein with 3 grams of BCAA, there were significant increases in bench press and leg press strength (Kerksick, et al., 2006). With the whey protein being added it is not known if the results were seen due to the increase levels of BCAAs or by the protein supplement itself.

On the other hand, there are other studies that seem to show no improvements at all in muscular strength. This was observed in a study using 25 competitive wrestlers for a 10-day testing period. The subjects in the study were ingesting a hypo-caloric diet that was either control, high in protein, high in BCAAs, or low in protein. At the end of the 19-day study, measurements were taken and while there was a decrease in visceral

adipose tissue there was no significant increase in muscular strength (Mourier, Bigard, de Kerviler, Roger, Legrand, & Guezennec, 1997). Being that this study was done with a hypo-caloric diet, it is not completely obvious if the lack of improvements in muscular strength were associated with BCAAs. By having a hypo-caloric diet, the body is already being placed in a catabolic state. With the body being in a catabolic state it is already breaking down skeletal muscle for energy and, therefore, as long as there was no loss in muscular strength there were some effects being seen. It was seen that while in a fasting state there were increased levels of BCAAs, and that when in this fasting state there is an increase in BCAA catabolism, causing unreliable results (Tom & Nair, 2006). Because of this, it is unknown if the results that were found were caused by the subjects being in a fasting state during the study or if it was because of the lack of BCAA reaction in the body.

CHAPTER THREE

Materials and Methods

Participants

Seven healthy, resistance trained [regular, consistent resistance training (i.e. thrice weekly) for at least one year prior to the onset of the study], males between the ages of 18-35 and a body mass index between 18.5-30 kg/m^2 participated in the double-blind study. Enrollment was open to men of all ethnicities. Only participants considered as low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM) and who had not consumed any nutritional supplements (excluding a daily multi-vitamin) 3 months prior to the study were allowed to participate. All participants provided written informed consent and were cleared for participation by passing a mandatory medical screening. All eligible subjects signed university-approved informed consent documents and approval was granted by the Institutional Review Board for Human Subjects. Additionally, all experimental procedures involved in the study conformed to the ethical consideration of the Helsinki Code.

Study Site

All supervised testing and supplement assignment was conducted in the Exercise and Sport Nutrition Laboratory at Baylor University.

Baseline and Familiarization

Participants expressing interest in participating in this study were interviewed on the phone to determine whether they appeared to qualify to participate in this study. Participants believed to meet eligibility criteria were then invited to attend an entry/familiarization session. Once reporting to the lab, participants completed a medical history questionnaire and underwent a general physical examination to determine whether they meet the eligibility criteria. Participants meeting entry criteria were familiarized to the study protocol via a verbal and written explanation outlining the study design and were then given an appointment time to perform baseline/pre-supplementation assessments. At this time, participants were instructed to refrain from exercise for 48 hours and record their dietary intake for 4 days prior to baseline testing. Participants were told to record all caloric food or beverages that were ingested during this 4 day period prior to baseline testing.

Body Composition

Height was measured using standard anthropometry. Total body weight was measured using a calibrated electronic scale with a precision of +/- 0.02 kg. Total body water was then estimated using a Xitron 4200 Bioelectrical Impedance Analyzer (San Diego, CA) which measures bio-resistance of water and body tissues based on a minute low energy, high frequency current (500 micro-amps at a frequency of 50 kHz) transmitted through the body. This is measured through four electrodes placed on the body: one electrode was placed on the posterior surface of the right wrist, in between the radial and ulna styloid processes (wrist bones), another electrode was placed on the posterior surface of the right hand at the distal base of the second metacarpal; the third

electrode was placed on the anterior surface of the right foot at the distal end of the first metatarsal. Participants lay on a table in the supine position and electrodes were connected to the analyzer for approximately 30 seconds.

Body composition was determined using a calibrated Hologic 4500W dual-energy x-ray absorptiometry (DEXA). The DEXA body composition test involved having the participant lie down on their back in a standardized position in a pair of shorts/t-shirt. A low dose of radiation then scanned their entire body for approximately six minutes. The DEXA segmented regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments for determination of fat, soft tissue (muscle), and bone mass.

Muscle Strength and Endurance Assessments

In order to determine possible effects of the supplement on upper- and lower-body muscular strength, participants performed one-repetition maximum (1-RM) tests on the free-weight bench press and angled leg press exercises prior to the first dose of supplement and beginning of the resistance-training program and after eight weeks of supplementation and resistance training. Participants warmed up by completing 5 to 10 repetitions at approximately 50% of the estimated 1-RM. The participant rested for 1 minute, and then completed 3 to 5 repetitions at approximately 70% of the estimated 1-RM. The weight was then increased conservatively, and the participant attempted to lift the weight for one repetition. If the lift was successful, the participant rested for 2 minutes before attempting the next weight increment. This procedure was continued until the participant failed to complete the lift. The 1-RM was recorded as the maximum weight that the participant was able to lift for one repetition. In order to assess muscle

endurance, using the bench press and angled leg press exercises, participants performed as many repetitions as possible with 75% of their 1-RM.

Muscle Power Assessment

In order to determine possible effects of the supplement on lower-body muscular power, participants performed a test of peak isometric torque and average power prior to the first dose of supplement and beginning of the resistance-training program and after eight weeks of supplementation and resistance training. Participants underwent the initial/baseline leg strength test to determine peak isometric torque and average power on the dominant leg with a knee extension (concentric)/flexion (concentric) protocol using a Biodex-System 3 (Biodex Medical Systems, Inc., NY, USA). The assessment session consisted of participants performing three sub-maximal trial repetitions at an estimated effort of 25%, 50%, 75%, and two maximal (100% effort) repetitions, a rest period of one minute, followed by five, ten, and fifteen maximal (100% effort) concentric repetitions at 60, 180, and 360 degrees/second respectively. The contractions were performed over a range of motion of 1.04 rad (2.08-1.04 rad of flexion, where 3.14 rad is full extension). The peak isometric torque and average power measurements at 60 degrees/second were divided by the participants' body weight and used at the dependent measures of isokinetic muscle strength and power.

Supplementation Protocol

Participants were assigned an eight-week supplementation protocol consisting of the daily oral ingestion of either a supplement containing 9 grams of a BCAA supplement [L-leucine (4.5 grams), L-isoleucine (2.25 grams), L-valine (2.25 grams)], (AST Sport

Science, Colorado Springs, CO) and 42 grams/day of maltodextrose (AST Sport Science, Golden, CO) or 9 grams of placebo [guar gum (Nutrition for Optimum Wellness, Bloomingdale, IL)] and 42 grams of maltodextrose (AST Sport Science, Colorado Springs, CO). All supplements were mixed in 30 ounces of water and half of the total daily dosage was ingested 30 minutes prior to each exercise session and the remaining half was ingested no later than 30 minutes following each exercise session. The supplements were ingested 4 times per week on exercise days only. Supplementation compliance was monitored by participants returning empty containers of their supplement following the eight weeks of supplementation, and also by completing weekly a supplement compliance questionnaire.

Resistance Training Protocol

Participants engaged in a supervised, periodized heavy resistance-training program four days per week, split into two upper and two lower extremity workouts per week for a total of eight weeks. Prior to the workout, subjects performed a standardized series of stretching exercises. The participants then performed an upper body resistance-training program consisting of nine exercises (bench press, lat pull, shoulder press, seated rows, shoulder shrugs, chest flies, biceps curl, triceps press down, and abdominal curls) twice per week and a seven-exercise lower extremity program (leg press, back extension, step ups, leg curls, leg extension, heel raises, and abdominal crunches) performed twice per week. Participants performed 3 sets of 10 repetitions with as much weight as they could lift per set (typically 70 – 80% of 1RM). Rest periods between exercises and sets lasted no longer than 2 minutes. Resistance training sessions were conducted at the

Student Life Center (SLC) at Baylor University and participants were required to document their training progress in exercise diaries provided by study personnel.

Statistical Analyses

Statistical analyses were performed by utilizing a repeated-measure two-factor [treatment groups (2) x time point (2)] multivariate analysis of variance (MANOVA). The MANOVA was performed due to the likelihood of the dependent variables being related to one another. In addition, the use of a MANOVA analysis also reduces the risk of Type I errors, by controlling for alpha level that could result with the use of repeated analyses of variance (ANOVA). In addition, for all statistical analyses not meeting the sphericity assumption for the within-subjects analyses, a Huynh-Feldt correction factor was applied to the degrees of freedom in order to adjust (increase) the critical F-value to a level that would prevent the likelihood of committing a type I error. Where appropriate, follow-up testing to the MANOVA for each dependent variable was performed using ANOVA. All statistical procedures were performed using SPSS 16.0 software (Chicago, IL) and a probability level of ≤ 0.05 was adopted throughout.

CHAPTER FOUR

Results

Subject Demographics

Twenty-one participants were initially recruited for the study, completed the consent forms, and participated in the initial familiarization session. Of those twenty-one subjects, only seven successfully completed the eight weeks of the study. Table 1 presents the demographic information for each group. Table 2 presents the baseline means and standard deviations (±SD) for height, weight, and age of each group.

Table 1

Subject Baseline Demographics

	BCAA + Carbohydrate	
Category	*Mean*	*Standard Deviation*
N size	*3*	
Weight (kg)	*75.27*	*±7.73*
Height (cm)	*176.09*	*±10.56*
Age (years)	*21.33*	*±1.15*
	Carbohydrate	
N size	*4*	
Weight (kg)	*71.33*	*±4.88*
Height (cm)	*180.67*	*±7.36*
Age (years)	*20*	*±1.83*

Dietary Analysis

Four days prior to baseline and follow-up testing participants were required to record their four-day dietary intakes. The diet logs were then used to analyze the average caloric and macronutrient intake and to see if there were any changes in the participants'

dietary habits during their participation in this study. The amounts of total kilocalories, protein, carbohydrates, and fats were expressed relative to body weight and used for statistical analysis. There was no significant Group x Time interactions for kilocalories (p=0.602, effect=0.309), protein (p=0.969, effect size= 0.002), carbohydrates (p=0.800, effect size=0.072), and fats (p=0.504, effect size=0.518) indicating there to be no differences between groups. There was also no significant Time main effect observed for kilocalories (p=0.35, effect size=1.06), protein (p=0.508, effect size=0.508), carbohydrates (p=0.139, effect size=3.096), and fats (p=0.905, effect size= 0.016) indicating no changes in dietary variables over the course of the eight weeks. It should be noted, that all participants were self-reporting their dietary intake over these four-day periods, and had no previous experience with the recording of dietary intake except for the instructions they were given during the initial familiarization session. As a result, this could have resulted in error with the reporting of the dietary intake.

Table 2

Dietary Intake

BCAA + Carbohydrate				
Groups	Total Calories (kcal/kg)	Carbohydrate (g/kg)	Protein (g/kg)	Fat (g/kg)
Pre-test	37.23 (±8.91)	4.68 (±1.39)	1.40 (±.23)	1.47 (±.34)
Post-test	31.57 (±3.84)	3.80 (±.35)	1.25 (±.015)	1.31 (±.38)
Carbohydrate				
Pre-test	29.65 (±9.98)	3.52 (±1.19)	1.24 (±.49)	1.16 (±.47)
Post-test	31.34 (±8.47)	4.17 (±.92)	1.338 (±.38)	1.05 (±.48)

Note: Data are represented as means (±SD)

Anthropometric Measures

Bodyweight

For bodyweight, there was no significant Group x Time interaction (p=.0.171, effect size= 2.55), nor was there a significant Time main effect (p=.493, effect size=0.546). Collectively, these results indicate the neither group underwent significant changes in bodyweight over the eight weeks of training and supplementation. Table 3 presents the means (±SD) for each group at the pre-test and post-test session.

Table 3

Bodyweight

	BCAA + Carbohydrate	
Test	Body Weight (kg)	Standard Deviation
Pre-test	75.27	±7.73
Post-test	76.87	±7.50
	Carbohydrate	
Pre-test	71.33	±4.89
Post-test	71.92	±2.62

Note: Data are represented as means (±SD)

Total Body Water

For total body water, there was no significant Group x Time interaction (p=0.107, effect size=3.863). There was also no Time main effect observed (p=0.992, effect size= 0.000) indicating that neither group underwent significant changes in body water over the eight weeks of training and supplementation. Table 4 presents the means (±SD) for each group at the pre-test and post-test session.

Table 4

Total Body Water

	BCAA + Carbohydrate	
Test	Body Water (L)	Standard Deviation
Pre-test	42.11	±8.31
Post-test	42.87	±8.52
	Carbohydrate	
Pre-test	40.82	±2.17
Post-test	41.56	±1.52

Note: Data are represented as means (±SD)

Fat-Free Mass

Fat-free mass produced no significant Group x Time interaction (p=0.063, effect size=5.646) or a Time main effect (p=0.426, effect size=0.751), which indicates that neither group underwent significant changes in fat-free mass over the eight weeks of training and supplementation. Therefore, hypothesis six, which states that eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly increase fat-free mass when compared to CHO, failed to be rejected. Table 5 presents the means (±SD) for each group at the pre-test and post-test session.

Fat Mass

For fat mass, there was no significant Group x Time interaction (p=0.825, effect size= 0.055) or any Time main effect (p=0.523, effect size= 0.471). These results indicate that neither group underwent significant changes in fat mass over the eight weeks of training and supplementation. Therefore, hypothesis seven, which states that eight weeks of resistance training combined with CHO and BCAA supplementation will

not significantly increase fat mass when compared to CHO, failed to be rejected. Table 5 presents the means (±SD) for each group at the pre-test and post-test session.

Table 5

Fat and Fat-Free Mass

	BCAA + Carbohydrate	
Test	Fat Mass (kg)	Fat-Free Mass (kg)
Pre-test	12.19 (±4.38)	58.72 (±8.83)
Post-test	12.00 (±3.49)	60.48 (±7.54)
	Carbohydrate	
Pre-test	9.33 (±2.03)	57.59(±4.36)
Post-test	9.72 (±1.30)	58.40 (±3.30)

Note: Data are represented as means (±SD)

Muscle Strength

For upper-body strength, there was not a significant Group x Time interaction (p=0.814, effect size=0.062); however, there was a significant Time main effect (p=0.012, effect size= 14.533). These results indicate that both groups underwent significant changes in upper-body strength over the eight weeks of training and supplementation that were not different from each other. Therefore, hypothesis one, which states that eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly increase upper-body strength when compared to CHO, failed to be rejected. Table 6 presents the means (±SD) for each group at the pre-test and post-test session.

Lower-Body Strength

For lower-body strength, a significant Group x Time interaction (p=0.259, effect size= 1.622) was not observed. However, there was a significant Time main effect (p=0.002, effect size=33.013). These results indicate that both groups underwent

significant changes in lower-body strength over the eight weeks of training and supplementation that were not different from each other. Therefore, hypothesis two, which states that eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly increase lower-body strength when compared to CHO, failed to be rejected. Table 6 presents the means (±SD) for each group at the pre-test and post-test session.

Table 6

Relative Muscle Strength

	BCAA + Carbohydrate	
Test	Upper-Body Strength (kg/kg)	Lower-Body Strength (kg/kg)
Pre-test	1.22 (±0.039)	5.06 (±0.50)
Post-test	1.34 (±0.14)	6.11 (±0.96)
	Carbohydrate	
Pre-test	1.07 (±0.150)	4.14 (±0.96)
Post-test	1.21 (±0.16)	5.79 (±0.56)

Note: Data are represented as means (±SD)

Muscle Endurance

Upper Body Muscle Endurance

For upper-body muscle endurance, there was no significant Group x Time interaction (p=0.721, effect size=0.143) and there was also no significant Time main effect (p=0.721, effect size=0.143). These results indicate that neither group underwent significant changes in upper-body muscle endurance over the eight weeks of training and supplementation. Therefore, hypothesis three, which states that eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly increase upper-body muscle endurance when compared to CHO, failed to be rejected. Table 7

present the mean values (±SD) for upper-body muscle endurance at the pre-test and post-test sessions.

Lower Body Muscle Endurance

There was a trend towards Group x Time interact (p=0.054, effect size=6.323) however, there was no Time main effect (p=0.683, effect size=0.188). These results indicate that neither group underwent significant changes in lower-body muscle endurance over the eight weeks of training and supplementation. Therefore, hypothesis four, which states that eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly increase lower-body muscle endurance compared to CHO, failed to be rejected. Table 7 presents the mean values (±SD) for lower-body muscle endurance at the pre-test and post-test sessions.

Table 7

Upper and Lower Body Endurance

	BCAA + Carbohydrate	
Test	Upper Body Endurance (reps)	Lower Body Endurance (reps)
Pre-test	8.33 (±1.53)	15.00 (±6.25)
Post-test	8.67 (±2.08)	20.67 (±1.15)
	Carbohydrate	
Pre-test	9.75 (±1.71)	17.50 (±6.25)
Post-test	9.75 (±2.36)	21.50 (±10.85)

Note: Data are represented as means (±SD)

Muscle Power

Muscle power for the dominant leg is presented as relative peak torque and average power at 60 degrees/sec and expressed relative to body weight. Table 8 presents

the mean values (±SD) for peak torque and average power at the pre-test and post-test sessions.

Peak Torque

For peak torque, there was no significant Group x Time interaction (p=0.865, effect size=0.032) and there was also no significant Time main effect (p=0.474, effect size=0.599) observed. These results indicate that neither group underwent significant changes in upper-body muscle endurance over the eight weeks of training and supplementation. Therefore, hypothesis five, which states that eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly increase lower-body muscle power when compared to CHO, failed to be rejected.

Table 8

Lower-Body Peak Torque and Average Power

	BCAA + Carbohydrate	
Test	Relative Peak Torque (ft-lbs/kg)	Relative Average Power (ft-lbs/kg)
Pre-test	1.77 (±0.29)	1.53 (±0.24)
Post-test	1.68 (±0.10)	1.63 (±0.07)
	Carbohydrate	
Pre-test	2.19 (±0.13)	1.87 (±0.17)
Post-test	2.25 (±0.21)	2.01 (±0.26)

Note: Data are represented as means (±SD). Relative peak torque and average power measurements performed at 60 degrees/sec.

Average Power

There was no significant Group x Time interaction (p=0.365, effect size=0.994) and there was also no significant Time main effect (p=0.866, effect size=0.032) observed, and indicate that neither group underwent significant changes in upper-body muscle

endurance over the eight weeks of training and supplementation. Therefore, hypothesis five, which states that eight weeks of resistance training combined with CHO and BCAA supplementation will not significantly increase lower-body muscle power when compared to CHO, failed to be rejected.

CHAPTER FIVE

Discussion

The purpose of this study was to examine the effect of BCAA and carbohydrate supplementation in conjunction with heavy resistance training over an eight week period. The supplements were taken 4 days a week for 8 weeks on the days of exercise to determine if BCAA supplementation had differential effects on body composition and muscle performance compared to carbohydrate. It was expected that there would have been an increase in muscle protein synthesis in both groups allowing for improvements in body composition and muscle performance (Liu Z. , Jahn, Long, Frybug, Wei, & Barrett, 2001) (Drummond, Dreyer, Fry, Glynn, & Rasmussen, 2009). However, at the completion of the eight week training period the participants did not show any significant improvements in any of the variables measured.

Body Composition

In regards to body composition, there was no significant increase in fat-free mass and no significant decrease in fat mass. In addition, there was no significant change in body water. The lack of changes in body water in the participants demonstrated that there was no major loss or gain of water content that could have had an effect on body weight between the testing sessions. The results of the current study are in disagreement with other studies in which there were increases in fat-free mass and muscle performance in those who supplemented with BCAAs (Candeloro, Bertini, Melchiorri, & De Lorenzo, 1995), and also demonstrated that with a combination of moderate energy restriction and

BCAA supplementation there was a significant decrease in the visceral adipose tissue (Mourier, Bigard, de Kerviler, Roger, Legrand, & Guezennec, 1997). In the current study, while there was an increase in fat-free mass and a decrease in fat mass seen with both the BCAA + carbohydrate and carbohydrate groups from pre-test to post-test, these changes were not signifcantly different. Both supplements have been reported in previous studies to cause an increase in MPS, thereby causing an increase in fat-free mass (Drummond, Dreyer, Fry, Glynn, & Rasmussen, 2009) (Shimomura, Murakami, Nakai, Nagasaki, & Harris, 2004). With the current study, an increase in fat free mass was expected due to the increases in MPS that have been noted in other studies. We had expected to see an exacerbated effect on MPS, thereby increasing fat-free mass in the BCAA + carbohydrate groups that have been observed in other studies (Bird, Tarpenning, & Marino, 2006) (Dreyer, et al., 2008). However, since fat-free mass was not enhanced due to supplementation, and there was also no significant decrease in fat mass, it is conceivable that there was no enhanced supplementation-induced MPS.

Muscle Strength, Power, and Endurance

Significant increases in upper- and lower-body muscle strength were observed in the current study, which is in agreement with previous studies (Mero, 1999) (Kerksick, et al., 2006); however, these improvements were not significantly different between the two groups. In a 10-week study utilizing whey protein + BCAA supplementation with heavy resistance training, significant increases in upper and lower body muscular strength were observed (Kerksick, et al., 2006). Based on this previous study, in the current study an increase in increase in muscular strength over the eight-week training period was expected. However, the lack of significant improvements between the two groups

indicates that increases in muscle strength were due to the heavy resistance training protocol rather than the supplementation protocol. The results for muscle strength in the current study are corroborated by a study that utilized competitive wrestlers that found BCAA supplementation had no increase in muscular strength (Mourier, Bigard, de Kerviler, Roger, Legrand, & Guezennec, 1997).

In the current study, there were no significant improvements observed for average power and peak torque. While there was a slight improvement seen in relative average power, there were no significant improvements seen between the groups or between tests. Data in the literature on isokinetic muscle power and peak torque does not appear to exist in regard to demonstrating any improvements with BCAA or carbohydrate supplementation, but the current study did not demonstrate that any significant improvements occurred.

The participants showed no improvements in upper- or lower-body endurance. There was, however, a strong trend toward improvements ($p=0.054$) in lower-body endurance between tests. However, the improvements observed in lower-body endurance between test times were likely due to the resistance protocol, rather than the supplementation regimen.

Considerations and Conclusions

BCAAs + Carbohydrate did not seem to show any improvements in muscle strength and performance compared carbohydrate. The BCAA + carbohydrate group and the carbohydrate group both showed similar results, indicating that any changes were most likely due to the resistance training protocol and not the supplementation protocol. There were no significant changes in body weight, fat-free mass or fat mass from pre-test

to post-test, and there were not any significant changes between groups. The lack of changes in body composition showed that there was a lack of MPS that had been noted in other studies (Candeloro, Bertini, Melchiorri, & De Lorenzo, 1995) (Drummond, Dreyer, Fry, Glynn, & Rasmussen, 2009) (Shimomura, Murakami, Nakai, Nagasaki, & Harris, 2004). Both the BCAA + carbohydrate group and the carbohydrate group seemed to maintain their body composition throughout the eight weeks of the study. The improvements that were observed among the participants were expected due to the nature of the resistance training protocol, since the participants were non-resistance trained. Even though there did not seem to be any significant improvements in the body composition, there were slight improvements observed in both groups fat mass and fat-free mass. Improvements in fat loss and fat-free mass gain are expected when untrained individuals begin a heavy resistance training protocol, such as the one utilized in the current study. There is a possibility that with a larger group of participants that there could have been more significant results; therefore, more testing is needed to determine if long-term benefits of BCAA supplementation could have more significant effects.

In comparison to the results seen in short term studies, such as increases in fat-free mass, decreases in fat mass, and increases in muscular strength (Blomstrand, Eliasson, Karlsson, & Kohnke, 2006) (Candeloro, Bertini, Melchiorri, & De Lorenzo, 1995) (Shimomura, et al., 2006) (Hong & Layman, 1984) (Kerksick, et al., 2006) (Rieu, et al., 2003), results from the current study failed to provide any corroboration. This could be due to the limited number of participants that completed the study, but with what was observed, we can only speculate that BCAA supplementation at the dose utilized does not improve body composition and muscle performance during eight weeks

of heavy resistance training. Because both groups had carbohydrates and both showed to have similar results on muscle performance it is possible that the improvements that were seen, while not significant, were possibly aided by the ingestion of the carbohydrate supplements. However, since there was no BCAA-only group involved in the current study, this statement must be interpreted with caution.

Because of the results from the current study, there appears to be no need for supplementing with BCAAs or carbohydrates during extended periods (i.e., eight weeks or longer) of heavy resistance training. Since most studies have shown improvements in muscle performance or body composition in single bout studies it might be suggested that there is not an enhanced effect associated with BCAA supplementation in conjunction with heavy resistance training.

APPENDICES

APPENDIX A

Medical History

BAYLOR UNIVERSITY
ESNL

Medical History Inventory

Directions. The purpose of this questionnaire is to enable the staff of the Exercise and Sport Sciences Laboratory to evaluate your health and fitness status. Please answer the following questions to the best of your knowledge. All information given is **CONFIDENTIAL** as described in the **Informed Consent Statement.**

Name:_____ Age: _____Date of Birth: _____

Name and Address of Your Physician:
_____

MEDICAL HISTORY

Do you have or have you ever had any of the following conditions? (Please write the date when you had the condition in blank).

___ Heart murmur, clicks, or other cardiac findings?
___ Frequent extra, skipped, or rapid heartbeats?
___ Chest Pain (with or without exertion)?
___ High cholesterol?
___ Diagnosed high blood pressure?
___ Heart attack or any cardiac surgery?
___ Leg cramps (during exercise)?
___ Chronic swollen ankles?
___ Varicose veins?
___ Frequent dizziness/fainting?
___ Muscle or joint problems?
___ High blood sugar/diabetes?
___ Thyroid Disease?
___ Low testosterone/hypogonadism?
___ Glaucoma?
___ Asthma/breathing difficulty?
___ Bronchitis/Chest Cold?
___ Melanoma/Skin Lesions?
___ Stroke or Blood Clots?
___ Emphysema/lung disease?
___ Epilepsy/seizures?
___ Rheumatic fever?
___ Scarlet fever?
___ Ulcers?
___ Pneumonia?
___ Anemias?
___ Liver or kidney disease?
___ Autoimmune disease?
___ Nerve disease?
___ Psychological Disorders?

Do you have or have you been diagnosed with any other medical condition not listed?
_____
_____

_____
_____

Please provide any additional comments/explanations of your current or past medical history.
_____
_____

Please list any recent surgery (i.e., type, dates etc.).
_____
_____
_____

List all prescribed/non-prescription medications and nutritional supplements you have taken in the last 3 months.

What was the date of your last complete medical exam?

Do you know of any medical problem that might make it dangerous or unwise for you to participate in this study (including strength and maximal exercise tests) ____ If yes, please explain:

Recommendation for Participation

____ No exclusion criteria presented. Subject is ***cleared*** to participate in the study.

____ Exclusion criteria is/are present. Subject is ***not cleared*** to participate in the study.

Signed: _____ Date: _____

APPENDIX B

Dietary Recall Form

Baylor University
Exercise & Sport Nutrition Laboratory

NAME _____ **Date** _____

INSTRUCTIONS

1. Record everything you eat for 4 days (including one weekend day). If you eat pretzels, record how many. If you eat a bag of chips, record the number of ounces. For drinks, record the number of cups or ounces. Record everything you drink except water.
2. Record the Food, Amount, Brand Name, and Preparation Methods. For example: baked vs. fried chicken; 1 cup of rice; 2 teaspoons of margarine; 1 cup of 2% milk; McDonald's, Healthy Choice, or Frosted Flakes.
3. Record immediately after eating. Waiting until that night may make it difficult to remember all foods and quantities.

Food (include brand)	Method of Preparation	Quantity (cups, oz., no.)
BREAKFAST:		
LUNCH:		

DINNER:

_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

SNACKS:

_____	_____	_____
_____	_____	_____
_____	_____	_____

APPENDIX C

IRB Report

Application to the Baylor IRB
For Review of Research/Activity Proposal

Part 1: Signature Page

1. Name Darryn S. Willoughby, Ph.D.

2. Email Address (optional) Darryn_Willoughby@baylor.edu

3. Complete Mailing Address P.O. Box 97313

4. Position Associate Professor

5. Faculty Advisor (if researcher is Graduate Student) _____

6. Department/School HHPR

7. Telephone # x3504 FAX # x3527

8. Are you using participants in research (***Y*** or N) or in teaching exercises (Y or ***N***)?

9. Title of the research project/teaching exercise:

The Effects of 10 Weeks of Heavy Resistance Training and Branched Chain Amino Acid Supplementation on Muscle Performance and Body Composition

10. Please return this signed form along with all the other parts of the application and other documentation to the University Committee for Protection of Human Subjects in Research; Dr. Matt Stanford, Chairman, Department of Psychology and Neuroscience, Baylor University, P.O. Box 97334, Waco, Texas 76798-7334. If you have questions, or if you would like to see a copy of the OHRP Report on protection of human subjects in research, contact Dr. Stanford at extension 2961.

_____ 10/05/10
Signature of Principal Investigator Date

_____
Signature of Faculty Advisor (required if researcher is a Graduate Student)

Departmental Review: _____
Department Chair or the Chair's Designate

Part 2. Introduction & Rationale

It is well known that resistance training can lead to skeletal muscle hypertrophy. Recently, scientists have been investigating the mechanisms behind such adaptations. In general, there are two major factors that promote protein synthesis: 1) DNA transcription of muscle specific genes (gene expression) and 2) the translation of these mRNA transcripts into functional proteins. Branched-chain amino acids (BCAA) make up about one third of skeletal muscle tissue within the human body and consist of leucine, isoleucine, and valine (1), and have been shown to increase translation of skeletal muscle proteins via its activation of the Akt/mTOR pathway (2). Of the three branched-chain amino acids, it appears that leucine plays the greatest role in promoting translation initiation. Specifically, leucine has been shown to activate mTOR and its downstream targets in direct fashion. Not only being a key component of muscle tissue, there seems to be some correlation between BCAAs and increases in mammalian target of rapamycin (mTOR) activation, 70-kDa ribosomal protein S6 kinase (p70S6K), and eukaryotic initiation factor 4E-binding protein 1 (eIF4E-BP1) have been shown to increase muscle protein synthesis (MPS), thereby leading to an increase in lean muscle mass and muscular strength. Both p70S6K and eIF4E-BP1 have been identified as key components to signaling pathways leading to MPS (3). This net anabolic effect is possibly related to the increases in mTOR, p70S6K, and eIF4E-BP1. BCAAs seem to cause this effect by phosphorylating mTOR, which causes a sequential activation of p70S6K and eIF4E-BP1 and increases the translation of specific mRNA (4).

Based on the mechanisms in which BCAAs appears to up-regulate muscle protein synthesis, there are many claims of BCAAs improving muscular strength and mass when combined with resistance training; however, there is little scientific research to substantiate these claims. The increases in lean muscle mass that occur with resistance training are typically correlated with improvements in muscular strength. In human skeletal muscle BCAAs, act directly as a nutrient signal to activate mRNA expression and potentiate muscle protein synthesis (3). As a result, BCAAs taken as a supplement to resistance training may be able to produce a net ergogenic effect, thereby improving muscle performance. Moreover, the purpose of this study is to determine the effects of 10 weeks of heavy resistance training and BCAA supplementation on body composition (fat-free mass, fat mass, percent fat) and muscle performance (strength, power, endurance).

Part 3. Methodology

Participants

Forty-five apparently healthy, non-resistance trained [no regular, consistent resistance training (i.e. thrice weekly) for at least one year prior to the onset of the study] males

between the ages of 18-30 and a body mass index between 18.5-30 kg/m2 will volunteer to participate in the randomized, double-blind study. Enrollment will be open to men of all ethnicities. Only participants considered as low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM) and who have not consumed any nutritional supplements (excluding multi-vitamins) 3 months prior to the study will be allowed to participate. All participants must provide written informed consent and be cleared for participation by passing a mandatory medical screening. All eligible subjects will sign university-approved informed consent documents and approval will be granted by the Institutional Review Board for Human Subjects. Additionally, all experimental procedures involved in the study will conform to the ethical consideration of the Helsinki Code.

Study Site

All supervised testing and supplement assignment will be conducted in the Exercise & Sport Nutrition Laboratory and Exercise and Biochemical Nutrition Laboratory at Baylor University.

Independent and Dependent Variables

Table 1 shows the general research design protocol that will be administered in this study. The independent variable will be the ingestion of nutritional supplements comprising of BCAAs, a "comparator", or placebo. Dependent variables evaluated will be body composition (fat-free mass, fat mass, percent fat) and muscle performance (strength, power, endurance).

Entry and Familiarization

Participants expressing interest in participating in this study will be interviewed on the phone to determine whether they appear to qualify to participate in the study. Participants believed to meet eligibility criteria will then be invited to attend an entry/familiarization session. Once reporting to the lab, participants will complete a medical history questionnaire and undergo a general physical examination to determine whether they meet eligibility criteria. Participants meeting entry criteria will be familiarized to the study protocol via a verbal and written explanation outlining the study design and will then be given an appointment time to perform baseline/pre-supplementation assessments. At this time, participants will be instructed to refrain from exercise for 48 hours and record their dietary intake for 4 days prior to baseline testing which will include the assessment of body composition and muscle strength, endurance, and power.

Body Composition

In order to determine possible effects of the supplement on body composition, total body weight, total body water, fat-free mass, fat mass, and percent fat will be assessed before the first dose of supplement and beginning of the resistance training program and after 10 weeks of supplementation and resistance training. Based on our previous guidelines

(5,6), height will be measured using standard anthropometry. Total body weight will be measured using a calibrated electronic scale with a precision of +/- 0.02 kg. Total body water will then be estimated using a Xitron 4200 Bioelectrical Impedance Analyzer (San Diego, CA) which measures bio resistance of water and body tissues based on a minute low energy, high frequency current (500 micro amps at a frequency of 50 kHz) transmitted through the body. This is measured through four electrodes placed on the body: one electrode will be placed on the posterior surface of the right wrist, in between the radial and ulna styloid processes (wrist bones), another electrode will be placed on the posterior surface of the right hand at the distal base of the second metacarpal; the third electrode will be placed on the anterior surface of the right foot at the distal end of the first metatarsal. Participants will lie on a table in the supine position and electrodes will be connected to the analyzer for approximately 30 seconds.

Body composition will then be determined using a calibrated Hologic 4500W dual energy x ray absorptiometry (DEXA). The DEXA body composition test will involve having the participant lie down on their back in a standardized position in a pair of shorts and t-shirt. A low dose of radiation will then scan their entire body for approximately six minutes. The DEXA segments regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments for determination of fat, soft tissue (muscle), and bone mass.

Muscle Strength and Endurance Assessments

In order to determine possible effects of the supplement on muscular strength, participants will perform one-repetition maximum (1-RM) tests on the free-weight bench press and angled leg press exercises prior to the first dose of supplement and beginning of the resistance training program and after 10 weeks of supplementation and resistance training. Based on our previous guidelines (5,6,7), participants will warm up by completing 5 to 10 repetitions at approximately 50% of the estimated 1-RM. The subject will rest for one minute, and then complete three to five repetitions at approximately 70% of the estimated 1-RM. The weight will then be increased conservatively, and the subject will attempt to lift the weight for one repetition. If the lift is successful, the subject will rest for two minutes before attempting the next weight increment. This procedure will be continued until the subject fails to complete the lift. The 1-RM will be recorded as the maximum weight that the subject is able to lift for one repetition. In order to assess muscle endurance, using the bench press and angled leg press exercises, participants will perform as many repetitions as possible will 75% of their 1-RM.

Muscle Power Assessment

In order to determine possible effects of the supplement on muscular power, participants will perform a test of peak power prior to the first dose of supplement and beginning of the resistance training program and after 10 weeks of supplementation and resistance training. Based on our previous guidelines (8), participants will undergo the initial/baseline leg strength test to determine peak power on the dominant leg with a knee extensor protocol using a Biodex-System 3 (Biodex Medical Systems, Inc., NY, USA).

The assessment session will consist of participants performing three sub-maximal trial repetitions at an estimated effort of 25%, 50%, 75%, and two maximal (100% effort) repetitions, a rest period of one minute, followed by five maximal (100% effort) concentric repetitions at 60, 180, and 360 degrees/second. The contractions will be performed over a range of motion of 1.04 rad (2.08-1.04 rad of flexion, where 3.14 rad is full extension).

Resistance Training Protocol

Based on our previous guidelines (5,6,7), participants will engage in a supervised, periodized four-day per week resistance training program split into two upper and two lower extremity workouts per week for a total of 10 weeks. Prior to the workout, subjects will perform a standardized series of stretching exercises. The participants will then perform an upper body resistance-training program consisting of nine exercises (bench press, lat pull, shoulder press, seated rows, shoulder shrugs, chest flies, biceps curl, triceps press down, and abdominal curls) twice per week and a seven exercise lower extremity program (leg press, back extension, step ups, leg curls, leg extension, heel raises, and abdominal crunches) performed twice per week. Participants will perform 3 sets of 10 repetitions with as much weight as they can lift per set (typically 70 – 80% of 1RM). Rest periods between exercises and sets will last no longer than two minutes. Resistance training will be conducted at the Student Life Center (SLC) at Baylor University and participants will be required to document their training progress in exercise diaries provided by study personnel.

Supplementation Protocol

Participants will be assigned a 10-week supplementation protocol consisting of the daily oral ingestion of either a supplement containing 4.5 grams of BCAA [L-leucine (2.25 grams), L-isoleucine (1.125 grams), L-valine (1.125 grams), AST Sport Science, Colorado Springs, CO), 30 grams of maltodextrose (AST Sport Science, Colorado Springs, CO), 4.5 grams of BCAA plus 30 grams of maltodextrose, and a color and flavor-matched placebo containing sucralose (Crystal Light™) . Supplements will be mixed in 30 ounces of water and half of the total daily dosage will be ingested 30 minutes prior to each exercise session and the remaining half will be ingested no later than 30 minutes following each exercise session. The supplements will be ingested 4 times per week on exercise days only. Supplementation compliance will be monitored by participants returning empty containers of their supplement following the 10 weeks of supplementation, and also by completing weekly a supplement compliance questionnaire.

Reported Side Effects from Supplements

Before and after the 10-week supplementation period, participants will report by questionnaire whether they tolerated the supplement, supplementation protocol, as well as report any medical problems/symptoms they may have encountered throughout the protocol of the study.

Statistical Analyses

Statistical analyses will be performed by utilizing a repeated-measure two-factor [treatment groups (3) x time point (2)] mixed methods multivariate analysis of variance (MANOVA). The MANOVA will be performed due to the likelihood of the dependent variables being related to one another. In addition, the use of a MANOVA analysis also reduces the risk of Type I errors, by controlling for alpha level that could result with the use of repeated analyses of variance (ANOVA). In addition, for all statistical analyses not meeting the sphericity assumption for the within-subjects analyses, a Huynh-Feldt correction factor will be applied to the degrees of freedom in order to adjust (increase) the critical F-value to a level that would prevent the likelihood of committing a type I error. Where appropriate, follow-up testing to the MANOVA for each dependent variable will be performed using ANOVA. Significant differences in mean values for main effects or interactions will be determined using a Tukey post-hoc test. All statistical procedures will be performed using SPSS 16.0 software (Chicago, IL) and a probability level of ≤ 0.05 was adopted throughout.

References

1. Mero A. Leucine supplementation and intensive training. *Sports Med*., 27:347-58, 1999.

2. Karlsson H, Nilsson P, Nilsson J, Chibalin A, Zierath J, Blomstrand E. Branched-chain amino acids increase p70S6k phosphorylation in human skeletal muscle after resistance exercise. Am J Physiol Endocrinol Metab. 287:E1-7, 2004.

3. Lui Z, Jahn L, Long W, Fryburg D, Wei L, Barrett E. Branched chain amino acids activate messenger ribonucleic acid translation regulatory proteins in human skeletal muscle, and glucocorticoids blunt this action. *J Clin Endocrinol Metab*. 86:2136-43, 2001.

4. Blomstrand E, Eliasson J, Karlsson H, Kohnke R. Branched-chain amino acids activate keyenzymes in protein synthesis after physical exercise. *J Nutr*. 136:269S-73S, 2006.

5. Shelmadine, B., Buford, T., Hudson, G., Redd, L., Cooke, M., Leutholtz, B., & Willoughby, D.S. Effects of 28 days of NO-Shotgun supplementation on body composition, muscle strength and mass, markers of satellite cell activation, and clinical safety markers in males. *Journal of the International Society of Sports Nutrition.* 6:16, 2009.

6. Spillane, M., Schoch, R., Cooke, M., Harvey, T., Greenwood, M., Kreider, R., & Willoughby, D.S. The effects of creatine ethyl ester supplementation combined with heavy resistance training on body composition, muscle performance, and serum and

muscle creatine levels. *Journal of the International Society of Sports Nutrition.* 6:6, 2009.

7. Willoughby D. S., Stout, J., & Wilborn C. Effects of heavy resistance training and timed ingestion of a protein and amino acid supplementation on muscle strength and mass and muscle anabolism. *Amino Acids*, 32(4), 467-478, 2007.

8. Kerksick, C., Kreider, R., & Willoughby, D.S. Intramuscular adaptations to eccentric exercise and antioxidant supplementation. *Amino Acids*. 39:219-32, 2010.

Research Team

Darryn S. Willoughby, Ph.D. Dr. Willoughby is Associate Professor of Exercise and Muscle Physiology and Biochemistry in the Department of Health, Human Performance, and Recreation at Baylor University, and also an Associate Professor in the Baylor Biomedical Science Institute. Dr. Willoughby is an internationally recognized exercise biochemist and molecular physiologist. He has conducted a vast amount of research focusing on the biochemical and molecular regulatory mechanisms regarding exercise performance and nutrition. Dr. Willoughby is the principal investigator and will maintain complete oversight of the study.

Paul LaBounty, Ph.D. Dr. La Bounty is an Assistant Professor of Exercise Physiology in the Department of Health, Human Performance, & Recreation at Baylor University. Dr. LaBounty will assist in providing oversight, in data collection, and performing laboratory analyses.

Brian Leutholtz, Ph.D. Dr. Leutholtz is a Professor of Exercise Physiology in the Department of Health, Human Performance, & Recreation at Baylor University. Dr. Leuholtz will assist in providing oversight, in data collection, and performing laboratory analyses.

Sean Foster, B.S. Mr. Foster is a student pursuing a Master's degree in Exercise Physiology in the Department of Health, Human Performance, & Recreation at Baylor University. Mr. Foster will assist in data collection, and performing laboratory analyses.

Procedures

Medical Monitoring. Interested participants will be invited to familiarization sessions. During this time, participants will sign consent forms and complete medical history information. Participants will then undergo a general exam to determine whether the subject meets entry criteria to participate in the study. This exam will include evaluating the medical and training history questionnaires and performing a general physical examination according to ACSM exercise testing guidelines. Based on this examination, participants will be assessed for their risk of cardiovascular disease and contraindications

to exercise and then a recommendation will be made on whether the participant meets entry criteria and may therefore participate in the study. Trained, non-physician exercise specialists certified in CPR will supervise participants undergoing testing and assessments. A telephone is in the laboratory in case of any emergencies, and there will be no less than two researchers working with each participant during testing sessions. In the event of any unlikely emergency one researcher will check for vital signs and begin any necessary interventions while the other researcher contacts Baylor's campus police at extension 2222. Instructions for emergencies are posted above the phone in the event that any other research investigators are available for assistance. Participants will be informed to report any unexpected problems or adverse events they may encounter during the course of the study to Darryn S. Willoughby, Ph.D. If clinically significant side effects are reported, the participants will be referred to discuss the problem with their personal physician for medical follow-up. New findings and/or medical referrals of unexpected problems and/or adverse events will be documented, placed in the participants research file, and reported to the Baylor IRB committee.

Screening for Cardiopulmonary Disease Risk and Exercise Contraindications. All participants will have their risk of cardiopulmonary disease in accordance to standard procedures described by the American College of Sports Medicine (ACSM) (*ACSM's Guidelines for Exercise Testing and Prescription, 6th ed. Williams & Wilkins Publishers, 2000)*. Only those participants considered as low risk for cardiovascular disease will be considered as eligible to participate in the study. These guidelines are outlined and presented below:

ACSM Risk Stratification Criteria for Cardiovascular Disease

Low Risk

Younger individuals (men < 45 years of age; women < 55 years of age) who are asymptomatic for cardiovascular disease and possess no more than one positive cardiovascular disease risk factor.

Moderate Risk

Older individuals and/or those who are asymptomatic for cardiovascular disease and possess two or more cardiovascular disease risk factors.

High Risk

Individuals with one or more signs/symptoms suggestive or cardiovascular disease.

ACSM Criteria for Signs and Symptoms Suggestive of Cardiovascular Disease

1. Pain, discomfort in the chest, neck, jaw, arms, or other areas that may be due to myocardial ischemia.
2. Shortness of breath at rest or with mild exertion.
3. Dizziness or syncope.
4. Orthopnea or paroxysmal nocturnal dyspnea.
5. Ankle edema.

6. Palpitations or tachycardia.
7. Intermittent claudication.
8. Known heart murmur.
9. Unusual fatigue or shortness of breath with usual activities.

ACSM Absolute and Relative Contraindications to Exercise

Absolute Contraindications
1. Unstable angina.
2. Uncontrolled dysrhythmias.
3. Recent EKG changes and cardiac events.
4. Acute myocarditis or pericarditis.
5. Acute pulmonary embolism or acute myocardial infarction.
6. Severe aortic stenosis.
7. Dissecting aneurysm.
8. Acute infections.

Relative Contraindications
1. Left main coronary stenosis.
2. Severe hypertension (> 200/110).
3. Tachycardia or bradycardia.
4. Uncontrolled metabolic disease.
5. High-degree AV block.
6. Chronic infectious disease.
7. Cardiomyopahty and outflow obstructions.
8. Stenotic valve disease.
9. Ventricular aneurysm.

Assessment of Hemodynamic Safety Markers (Heart Rate & Blood Pressure). Heart rate will be determined by palpation of the radial artery using standard procedures. Blood pressure will be assessed in the supine position after resting for 5-min using a mercurial sphygmomanometer using standard procedures.

Reported Side Effects from Supplement Questionnaires. Participants will report by questionnaire whether they tolerated the supplement, supplementation protocol, as well as report any medical problems/symptoms they may have encountered throughout the protocol of the study.

Estimated Energy Intake/Dietary Inventories. Participants will record all food and fluid intake for 4 days on dietary record forms for dietary analysis in order to standardize nutritional intake. Participants will brings these forms with them upon each visit to the laboratory for the two testing sessions. Dietary intake will be assessed using the Food Processor IV Nutrition Software.

Body Composition Assessments. Participants will undergo body composition tests in the ESNL. Prior to each assessment, height will be measured using standard

anthropometry and total body weight will be measured using a calibrated electronic scale with a precision of +/- 0.02 kg. Total body water will then be estimated using a Xitron 4200 Bioelectrical Impedance Analyzer (San Diego, CA) which measures bio resistance of water and body tissues based on a minute low energy, high frequency current (500 micro amps at a frequency of 50 kHz) transmitted through the body. This analyzer is commercially available and has been used in the health care/fitness industry as a means to assess body composition and body water for over 20 years. The use of this device has been approved by the Food and Drug Administration (FDA) to assess total body water and the current to be used has been deemed safe. This is measured through four electrodes placed on the body: one electrode will be placed on the posterior surface of the right wrist, in between the radial and ulna styloid processes (wrist bones), another electrode will be placed on the posterior surface of the right hand at the distal base of the second metacarpal; the third electrode will be placed on the anterior surface of the right foot at the distal end of the first metatarsal. Participants will lie on a table in the supine position and electrodes will be connected to the analyzer. After the subject is connected, age, gender, weight, height, and activity level are entered into the unit by the technician. After the unit has measured the resistance, which takes approximately 30 seconds, the unit then calculates total body water and body water percent.

Body composition/bone density will then be determined using a calibrated Hologic 4500W dual energy x ray absorptiometry (DEXA). The DEXA body composition test will involve having the participant lie down on their back in a standardized position in a pair of shorts/t-shirt or a gown. A low dose of radiation will then scan their entire body for approximately six minutes. The DEXA segments regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments for determination of fat, soft tissue (muscle), and bone mass. Radiation exposure from DEXA for the whole body scan is approximately 1.5 mR per scan. This is similar to the amount of natural background radiation a person would receive in one month while living in Waco, TX. The maximal permissible x-ray dose for non-occupational exposure is 500 mR per year. Total radiation dose will be less than 5 mR for the entire study.

Muscle Strength Assessment. Participants will perform four one-repetition maximum (1-RM) tests on the bench press and angled leg press exercises. Participants will warm up by completing 5 to 10 repetitions at approximately 50% of the estimated 1-RM. The subject will rest for 1 minute, and then complete 3 to 5 repetitions at approximately 70% of the estimated 1-RM. The weight will then be increased conservatively, and the subject will attempt to lift the weight for one repetition. If the lift is successful, the subject will rest for 2 minutes before attempting the next weight increment. This procedure will be continued until the subject fails to complete the lift. The 1-RM will be recorded as the maximum weight that the subject is able to lift for one repetition.

Muscle Endurance Assessment. Using the bench press and angled leg press exercises, participants will perform as many repetitions as possible with 75% of their 1-RM.

Muscle Power Assessment. Participants will undergo the initial/baseline leg strength test to determine peak power on the dominant leg with a knee extensor protocol using a

Biodex-System 3 (Biodex Medical Systems, Inc., NY, USA). The assessment session will consist of participants performing three sub-maximal trial repetitions at an estimated effort of 25%, 50%, 75%, and two maximal (100% effort) repetitions, a rest period of one minute, followed by five maximal (100% effort) concentric repetitions at 0.52 rad s^{-1} (3). The contractions will be performed over a range of motion of 1.04 rad (2.08-1.04 rad of flexion, where 3.14 rad is full extension).

Equipment

Digital Scale. Total body weight will be determined using a digital scale accurate to ±0.02 kg. The scale is calibrated by placing certified 25-kg weights and balancing the scale. Other than general instructions, special skills are not required to measure body weight.

Mercurial Sphygmomanometer. Blood pressure will be assessed by auscultation of the brachial artery using a mercurial sphygmomanometer using standard clinical procedures.

Bioelectrical Impedance Analyzer (BIA). The Omron HBF-306 Bioelectrical Impedance Analyzer (Omron Healthcare Inc., Vernon Hills, IL) which measures bio-resistance and body composition based on a minute low energy, high frequency current transmitted through the body from surface electrodes embedded in the handles of the unit. The analyzer is calibrated internally to a standard electrical current by pressing the calibration key located on the unit. A trained research assistant will perform this procedure.

Dual-Energy X-Ray Absorptiometer (DEXA). Body composition measurements will be determined by qualified personnel (in compliance with State Regulations) using a Hologic Discovery W dual energy x-ray absorptiometer (Waltman, MA). This system segments regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments (i.e., bone mass, fat mass, and fat-free/soft tissue mass). Quality control (QC) calibration procedures will be performed on a spine phantom (Hologic X-CALIBER Model DPA/QDR-1 anthropometric spine phantom) prior to each testing session. In addition, weekly calibration procedures will be performed on a density step calibration phantom.

Resistance Exercise Machines. Maximum strength (1-RM strength) tests will be performed on an angled leg press (Nebula Fitness, Inc., Versailles, OH) and free-weight bench press (Body Masters, Inc., Rayne, LA). Equipment and testing will be contained within the EBNL.

Participants

Recruitment

Forty-five apparently healthy, non-resistance trained [no regular, consistent resistance training (i.e. thrice weekly) for at least one year prior to the onset of the study], males between the ages of 18-30 and a body mass index between 18.5-30 kg/m2 will volunteer

to participate in the double-blind study. Enrollment will be open to men of all ethnicities. A recruitment flyer will be posted on campus, and at area fitness centers.

Selection Criteria

Participants will not be allowed to participate in the study if they:

1. have not been involved in a habitual resistance training program (minimum of 3 hours/week for at least 1 year);
2. use tobacco products;
3. have orthopedic limitations that would limit participation in resistance training;
4. have any known metabolic disorder including heart disease, arrhythmias, diabetes, thyroid disease, or hypogonadism;
5. have a history of pulmonary disease, hypertension, hepatorenal disease, musculoskeletal disorders, neuromuscular/neurological diseases, autoimmune disease, cancer, peptic ulcers, anemia, or chronic infection (e.g., HIV);
6. are taking any heart, pulmonary, thyroid, anti-hyperlipidemic, hypoglycemic, anti-hypertensive, endocrinologic (e.g, thyroid, insulin, etc), emotional/psychotropic (e.g., Prednisone, Ritalin, Adderall), neuromuscular/neurological, or androgenic medications (anabolic steroids);
7. have taken ergogenic levels of nutritional supplements that may affect muscle mass (e.g.,creatine, HMB) or anabolic/catabolic hormone levels (e.g., androstenedione, DHEA, etc) within three months prior to the start of the study.
8. have any absolute or relative contraindication for exercise testing or prescription as outlined by the American College of Sports Medicine;
9. report any unusual adverse events associated with this study that in consultation with the supervising physician recommends removal from the study.

Compensation or Incentives

Participants completing all familiarization and testing sessions as well as turning in all required materials (i.e., dietary logs) in the study will be paid $100. Participants may receive information regarding results of these tests if they desire. This study does not constitute a teaching exercise and if participants are Baylor students, they will not receive any academic credit for participating in this study.

Potential Risks

The amino acids supplements to be investigated in this study have been studied for various exercise-related and medically-related benefits in humans. Furthermore, amino acid mixtures are often given to medical patients with the intent of helping to prevent muscle wasting. As a result, research has demonstrated that oral administration of this compound is not associated with any significant medical side effects. Moreover, this supplement is currently available in over the counter nutritional supplements sold in United States and Europe. As with the vast majority of nutritional supplements, however, the FDA has not evaluated the safety or marketing claims of over-the-counter amino acid supplements.

Subjects who meet eligibility criteria will be exposed to several muscle strength tests and a separate resistance exercise bout that may cause symptoms of fatigue, shortness of breath, and/or muscular fatigue/discomfort. The exercise tests may also cause short-term muscle soreness and moderate fatigue for several days following the tests. Subjects may also experience muscle strains/pulls during the exercise testing and/or training program. However, exercise sessions will be conducted by certified strength and conditioning specialists and monitored to ensure the subjects follow appropriate exercise guidelines. Potential risks of this study are as follows: acute musculoskeletal injury resulting from 1-RM testing and acute injury resulting from the exercise sessions. Injury due to 1- RM testing and exercise will be minimized by ensuring that all subjects adhere to correct lifting form while performing the exercise. In addition, only Dr. Willoughby and co-investigators of this study will conduct the testing and exercise procedures. Participants will be made aware of the intensity and duration of the expected soreness due to the exercise sessions. Even though the participants in this study will be physically active and healthy, and will be instructed to continue exercising, participants may also still experience short-term muscle soreness, moderate fatigue, and muscle strains/pulls during their routine resistance-training program.

Researchers involved in collecting data represent trained, non-physician, certified strength and conditioning specialists. All personnel involved in collecting data will be certified in CPR, which is also a condition to holding these professional certifications. A telephone and automated electronic defibrillator (AED) is located in the laboratory in case of any emergencies and there will be no less than two researchers working with each participant during testing. In the event of any unlikely emergency one researcher will check for vital signs and begin any necessary interventions while the other researcher contacts Baylor's campus police at extension 2222. Instructions for emergencies are posted above the phone in the event that any other research investigators are available for assistance.

Potential Benefits

The main benefit that participants may obtain from this study is that if nutritional supplements containing BCAAs are effective, there is a possibility that they may gain insight into how to possibly enhance muscle mass and performance with supplementation that typically occurs in conjunction with resistance training as well as improved health profiles. Participants may also gain insight about their health and fitness status from the assessments to be performed. However, even if no individual benefit is obtained, participating in this study will help to determine whether ingesting this nutritional supplement affects training adaptations. This information will be helpful to athletes and non-athletes alike who use nutritional BCAA supplements during training with the intent of improving subsequent muscular performance to know whether they are effective or not.

Assessment of Risk

Even though clinical data are available outlining the safety effects of many supplements containing BCAAs are still relatively new to the market, the potential exercise-related

benefits of BCAA supplement formulations are not yet well delineated. Although BCAAs are available in a number of over the counter nutritional supplements, initial results suggest that these supplements may provide benefit at increasing muscle strength and mass and enhancing training adaptations during training. However, additional well-controlled research is necessary before conclusions can be drawn. This study will help determine whether ingesting BCAAs in conjunction with resistance training effectively improves body composition and muscle for healthy, active, males. Consequently, the risk associated with BCAA supplementation to be evaluated in this study is low. The greatest risk associated with participating in this study will likely be from the muscle soreness participants will experience from participating in the resistance exercise protocol. However the intensity of the exercise protocol will be no more than when individuals engaged heavily in a new or different form of physical activity. Therefore, the potential benefits of subjects participating in this study outweigh the potential risks.

There is risk associated with participating in this study will be performing the strength and power testing and participating in the eight-week resistance training program. However, since the participants to be used in this study will all undergo medical screening, these risks would be no different than participating in their own exercise programs. Therefore, the potential benefits of participating in this study outweigh the potential risks.

Compensation for Illness or Injury

Each participant will agree to indemnify and hold harmless Baylor University, its officers, directors, faculty, employees, and students for any and all claims for any injury, damage or loss suffered as a result of participation in this study regardless of the cause of injury, damage, or loss.

Confidentiality

Information obtained from this research (including questionnaires, medical history, laboratory findings, or physical examination) will be kept confidential to the extent permitted by law. However, according to FDA regulations, records will be open to FDA representatives to review if necessary. This may include questionnaires, medical history, laboratory findings/reports, statistical data, and/or notes taken throughout this study. Records of the research may also be subpoenaed by court order or may be inspected by federal regulatory authorities. Data derived from this study may be used in reports, presentations and publications. Participants in this study will not be individually identified unless they give their written consent. All participants will have a number to identify their results. Only the study personnel will know the subject numbers. Only study personnel will have access to the data. All data will be stored in a locked cabinet in the Exercise and Biochemistry Laboratory and only Darryn Willoughby, Ph.D. will have access to the key. Additionally, that confidentiality will be maintained by assigning code numbers to my files, limiting access to data to research assistants, locking cabinets that store data, and providing passwords to limit access to computer files to authorized

personnel only. All evidence of primary data will be stored for exactly three years after the completion of the study. At this time data will be destroyed in a manner that instills complete privacy to all participants of the study.

Data Presentation & Publication

Data will be presented at an appropriate scientific conference (e.g., American College of Sports Medicine, International Society of Sports Nutrition, Experimental Biology, etc.) and published in a peer reviewed scientific journal (e.g., Medicine & Science in Sport and Exercise, Journal of Sport Science and Medicine, International Journal of Sport Nutrition and Exercise Metabolism, etc.).

Statement on Conflict of Interest

Supplements provided for this study were obtained from Scivation Inc. (Burlington, NC). Researchers involved in collecting data in this study have no financial or personal interest in the outcome of results or sponsors.

Table 1. Overview of Research Design

Visit 1 (Familiarization and Entry)	Visit 2 (Pre-Testing)	Visit 3 (Post-Testing)
Phone interview. Familiarization session. Informed Consent Form. Demographic Form. Health History Form. Activity Form. General Exam to Determine Qualifications to Participate in Study. Determination of Height and Body Weight. Determination of Resting Hemodynamic Measures (Heart Rate and Blood Pressure).	Baseline Hemodynamic Measures. Baseline DEXA for Body Composition. Baseline Muscle Strength, Endurance, and Power Assessments. Randomization Into Groups. Instructions for Supplementation Protocol. Baseline Diet Log Analysis.	Hemodynamic Measures. DEXA for Body Composition. Muscle Strength, Endurance, and Power Assessments. Diet Log Analysis. Training Log Analysis. Reported Side Effects from Supplement Questionnaire. Diet Log Analysis.

BAYLOR UNIVERSITY

Department of Health, Human Performance, & Recreation
Informed Consent Form

Title of Investigation: The Effects of 10 Weeks of Heavy Resistance Training and Branched Chain Amino Acid Supplementation on Muscle Performance and Body Composition

Principal Investigator: Darryn S. Willoughby, PhD
Associate Professor, Department of HHPR, Baylor University

Co-investigators: Paul LaBounty, PhD
Assistant Professor, Department of HHPR, Baylor University

Brian Leuholtz, PhD
Professor, Department of HHPR, Baylor University

Sean Foster, BS
Graduate Student, Department of HHPR, Baylor University

Sponsors: AST Sport Science, Inc. (Colorado Springs, CO)

Description of the Study

I will be one of 45 apparently healthy, non-resistance-trained males between the ages 18 to 30 who will participate in this study. I have not engaged in regular resistance training at least three times a week for one year. I understand that I will be required to visit the laboratory three times during the course of the study. During an initial familiarization session (visit 1), I will be informed of the requirements of the study and sign an informed consent statement in compliance with the Human Subjects Guidelines of Baylor University and the American College of Sports Medicine. A trained individual will examine me to determine if I am qualified to participate in this study. If I am cleared to participate in the study, I will be familiarized to the testing procedures. This session will take approximately 30 minutes to complete. Once I complete the familiarization session, I will be scheduled for baseline testing (visit 2). Following the familiarization session, I will be instructed to refrain from exercise for 48 hours and fast for 8 hours prior to baseline testing. I will be provided with a dietary analysis form that I am to complete for 4 days prior to baseline testing. Once I report to the lab for each testing session, I will turn in my dietary analysis form. I

understand that I will also return to the lab after the 10-week training and supplementation period for follow-up testing (visit 3).

I will be matched by my age and body weight and then randomly assigned in a double blind manner the daily oral ingestion of either a supplement containing 4.5 grams of BCAA [L-leucine (2.25 grams), L-isoleucine (1.125 grams), L-valine (1.125 grams), AST Sport Science, Colorado Springs, CO), 30 grams of maltodextrose (AST Sport Science, Colorado Springs, CO), 4.5 grams of BCAA plus 30 grams of maltodextrose, and a color and flavor-matched placebo containing sucralose (Crystal Light™) . Supplements will mixed in 30 ounces of water and half of the total daily dosage will be ingested 30 minutes prior to each exercise session and the remaining half will be ingested no later than 30 minutes following each exercise session. The supplements will be ingested 4 times per week on exercise days only for 10 weeks. Supplementation compliance will be monitored by me returning the empty containers of my supplement when I return to the lab for follow-up testing at visit 3, and also by completing a weekly supplement compliance questionnaire. I understand that if I do not take my supplements I will be removed from the study.

I understand that I will be required to participate in a periodized 4-day per week resistance-training program split into two upper and two lower extremity workouts per week for a total of 10 weeks. Prior to the workout, I will perform a standardized series of stretching exercises and then perform an upper body resistance-training program consisting of nine exercises (bench press, lat pull, shoulder press, seated rows, shoulder shrugs, chest flies, biceps curl, triceps press down, and abdominal curls) twice per week and a seven exercise lower extremity program (leg press or squat, back extension, step ups, leg curls, leg extension, heel raises, and abdominal crunches) performed twice per week. I understand that I will perform 3 sets of 10 repetitions with as much weight as I can lift per set (typically 70 – 80% of 1RM). I understand that my resistance training may be conducted at the Student Life Center (SLC) at Baylor University, but that I will be required to document my training progress on the form provided to me.

I understand that I will be required to report to the laboratory on visit 2 (prior to beginning the supplementation and resistance training protocol) to have my heart rate and blood pressure determined, to turn in my 4-day dietary records, have my body composition and muscle strength, endurance, and power determined. I understand that I will report back to the laboratory at the end of week eight (visit 3) to undergo the same testing procedures as I went through at visit 2; however, at this point I will also complete a report of side effects from supplementation questionnaire to determine if I have experienced any unexpected problems or adverse events from participating in this study. I understand that if clinically significant side effects are reported, I will be referred to discuss the problem with Darryn Willoughby, Ph.D. Upon his discretion, I may be referred to discuss the matter with my personal physician to determine whether any medical treatment is

needed and/or whether I can continue in the study. I understand that if I fail to report my progress and health status to the research assistant I may be removed from the study.

I agree to do my best to: 1) follow the instructions outline by the investigators; 2) show up to all scheduled testing times; and 3) take supplements as instructed. I agree not to take any other nutritional supplements or performance enhancing aids during this study (i.e. vitamins/minerals, creatine, HMB, androstenedione, DHEA, etc). In addition, I agree not to take any non-medically prescribed medications and to report any medication that is prescribed for me to take during this study. I understand that if I take any other nutritional supplements or medications during the course of the study that I will be removed from the study.

Exclusionary Criteria

I understand that in order to participate in the study, a trained individual will examine me to determine whether I qualify to participate. I understand that I will not be allowed to participate in this study if: 1.) I have any known metabolic disorder including heart disease, arrhythmias, diabetes, thyroid disease, or hypogonadism; 2.) I have a history of pulmonary disease, hypertension, liver or kidney disease, musculoskeletal disorders, neuromuscular or neurological diseases, autoimmune disease, cancer, peptic ulcers, or anemia; 3.) I am taking any heart, pulmonary, thyroid, anti-hyperlipidemic, hypoglycemic, anti-hypertensive, endocrinologic (ie, thyroid, insulin, etc), psychotropic, neuromuscular/neurological, or androgenic medications; 4.) I have any orthopedic limitations, 5.) I have any bleeding disorders; 6.) I have any chronic infections (e.g., HIV), 7.) I have taken any nutritional supplement, other than multi-vitamins, for three months prior to the study, 8.) I use tobacco products.

I have reported all nutritional supplements, medically prescribed drugs, and non-medically prescribed drugs that I am presently taking. I have reported whether I have had any prior allergic reactions to topical anesthetics. I have completed medical history questionnaires and am not aware of any additional medical problems that would prevent me from participating in this study. I agree to report all changes in medical status, nutritional and/or pharmacological agents (drugs) that I take during the course of the investigation to Darryn Willoughby, Ph.D. (254-710 3504). I understand that if I experience any unexpected problems or adverse events from participating in this study I may be referred to discuss the problem with my personal physician to determine whether any medical treatment is needed and/or whether I can continue in the study.

Risks and Benefits

I understand that even though clinical data are available outlining the safety effects of many BCAA supplements, the one used in this study is still relatively new to the market. Therefore, the potential benefits of the involved BCAA

supplement formulation are not yet well delineated. However, BCAA supplements are currently available in over the counter nutritional supplements sold in United States. As with any food or nutritional supplement, possible side effects may include stomach upset, gastrointestinal distress, allergic reactions, changes in mood and vigor, and/or changes in training adaptations. However, as with the vast majority of nutritional supplements, I understand that the FDA has not evaluated the safety or marketing claims of BCAA supplements. In addition, there are minor risks of muscular pain and soreness associated with the resistance training protocol required in this study which are not uncommon to any exercise program especially for individuals who do not resistance train on a regular basis.

I understand that the main benefits that may be obtained from this study are the potential ergogenic benefits of BCAA supplementation, which may include increased muscular strength, power, and reduced recovery time from resistance training.

Alternative Treatments

This is not a medical treatment. Therefore, if medical treatment is needed, I must obtain treatment for any medical problem I might have from my personal physician.

Costs and Payments

If I am a Baylor University student, I will not receive any academic credit for participating in this study. I understand that if I am an intercollegiate scholarship athlete I may not be eligible to receive payment to participate in this study. Eligible participants will be paid $100 for completing the familiarization and experimental testing sessions. I also understand that I may receive information regarding results of these tests if I desire.

New Information

Any new information obtained during the course of this research that may affect my willingness to continue participation in this study will be provided to me. In addition, I will be informed of any unusual/abnormal clinical findings in which medical referral to my personal physician may be warranted. If I desire, I may request that this information be provided to my physician.

Confidentiality

I understand that any information obtained about me in this research, including medical history, laboratory findings, or physical examination will be kept confidential to the extent permitted by law. However, I understand in order to ensure that FDA regulations are being followed, it may be necessary for a

representative of the FDA to review my records from this study which may include medical history, laboratory findings/reports, statistical data, and/or notes taken about my participation in this study. In addition, I understand that my records of this research may be subpoenaed by court order or may be inspected by federal regulatory authorities. I understand that data derived may be used in reports, presentations, and publications. However, I will not be individually identified unless my consent is granted in writing. Additionally, that confidentiality will be maintained by assigning code numbers to my files, limiting access to data to research assistants, locking cabinets that store data, and providing passwords to limit access to computer files to authorized personnel only. I understand that once blood and muscle samples are analyzed that they will be discarded.

Right to Withdrawal

I understand that I am not required to participate in this study and I am free to refuse to participate or to withdraw from the study at any time without penalty. Further, that my decision to withdraw from the study will not affect my care at this institution or cause a loss of benefits to which I might be otherwise entitled. If there is concern about my medical safety, I may be referred to seek medical attention.

Compensation for Illness or Injury

I understand that if I am injured as a direct result of taking part in this study, I should consult my personal physician to obtain treatment. I understand that the cost associated with the care and treatment of such injury will be the responsibility of me or my insurance carrier. In some cases, insurers may not reimburse claims submitted for a research-related injury resulting from medical procedures or treatments performed as part of a research study. I understand that Baylor University, the investigator's institutions, and the grant sponsor have not budgeted funds to compensate me for injury or illness that may result from my participation in this study and thus will not be accountable for illness or injury acquired during the course of this study. However, I may be referred to my personal physician if any clinically significant medical/psychological findings are observed during the course of this study.

I agree to indemnify and hold harmless Baylor University, its officers, directors, faculty, employees, and students for any and all claims for any injury, damage or loss I suffer as a result of my participation in this study regardless of the cause of my injury, damage or loss.

Statement on Conflict of Interest

I understand that the supplements for this study were donated by AST Sport Science, Inc. (Colorado Springs, CO). I understand that researchers involved in

collecting data in this study have no financial or personal interest in the outcome of results or sponsors.

Voluntary Consent

I certify that I have read this consent form or it has been read to me and that I understand the contents and that any questions that I have pertaining to the research have been, or will be answered by Darryn Willoughby, Ph.D. (principal investigator, Department of Health, Human Performance & Recreation, 120 Marrs McLean Gymnasium, Baylor University, phone: 254-710 3504) or one of the research associates. My signature below means that I am at least 18 years of age and that I freely agree to participate in this investigation. I understand that I will be given a copy of this consent form for my records. If I have any questions regarding my rights as a research subject in this study, I may contact Baylor's University Committee for Protection of Human Subjects in Research. The Chairman is Dr. Michael Sherr, School of Social Work, P.O. Box 97320, Waco, Texas 76798, phone number (254) 710-4483.

Date _____ Subject's Signature _____

I certify that I have explained to the above individual the nature and purpose of the potential benefits and possible risks associated with participation in this study. I have answered any questions that have been raised and have witnessed the above signature. I have explained the above to the volunteer on the date stated on this consent form.

Date _____ Investigator's Signature _____

Informed Consent Form Checklist

When using humans as participants in research you must obtain their informed consent. Check each of the following items as they appear on your Informed Consent Form and include this checklist with your protocol:

__x__ (a) A statement explaining the purpose of the research.

__x__ (b) A statement of the expected duration of the participant's participation.

__x__ (c) A description of the procedures to be followed.

__x__ (d) A description of any reasonable foreseeable risks or discomforts to the participant, including invasion of privacy.

__x__ (e) A description of any benefits resulting from the research, either to the participant or to others.

__x__ (f) A statement that informs participant of his/her right not to be a participant in a research project that is also a teaching exercise.

__x__ (g) A statement informing participant about how his/her anonymity will be guarded; i.e., that their confidentiality will be protected by assigned code numbers, by limitations of who has access to data, by data storage in locked cabinets, by locked computer files, etc.

__x__ (h) A statement that the participant's participation is voluntary, and that his/her refusal to participate will involve no penalty or loss of benefits to which the participant is otherwise entitled, and that the participant may discontinue participation at any time without penalty or loss of benefits to which the participant is otherwise entitled.

__na__ (i) A disclaimer, if applicable, regarding the use of the Internet to collect data.

__x__ (j) For research involving more than minimal risk, an explanation regarding the availability of any compensation or any medical treatments if injury occurs (if applicable, see OHRP Reports).

__x__ (k) If written informed consent is required, a place for the participant to sign and date the form and a statement that a copy of the signed consent form will be given to the participant for his/her records.

__na__ (l) If the participant is a minor, a statement of parental responsibility in consenting to the child's participation in the study with a place for the parent to sign and date the form in addition to the participant's signature.

__x__ (m) Include a short summary of your expertise related to this research proposal.

__x__ (n) The name, address, and telephone number of the principal investigator of the research project, and his/her affiliation with Baylor University. If the principal investigator is a graduate student, the name and telephone number of the faculty advisor is also required.

__x__ (o) A statement informing participant that inquiries regarding his/her rights as a participant, or any other aspect of the research as it relates to his/her participation as a participant, can be directed to Baylor's University Committee for Protection of Human Subjects in Research. The chairman is Dr. Matt Stanford, Professor Psychology and Neuroscience, PO Box 97334, Waco, Texas 76798-7334, phone number 254-710-2236.

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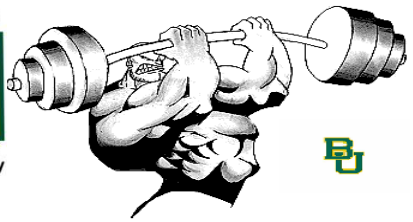

Want to get Paid to Workout?

Physically active Males Needed for a Weight Training & Branched-Chain Amino Acid Study

Researchers in the Exercise & Biochemical Nutrition Lab at Baylor University are recruiting 45 healthy and active, but non-resistance-trained men between the ages of 18-30 to participate in a study designed to evaluate the effects of a nutritional supplement containing branched-chain amino acids on body composition and muscle performance. Participants will be required to ingest supplements and engage in a 4-day/week resistance-training program for 10 weeks. Participants will be required to undergo testing for muscle strength, endurance, and power and body composition. Eligible subjects will receive $100 for completing the study and free supplement, muscle strength and body fat testing, and nutritional counseling.

For more information contact:

Exercise & Biochemical Nutrition Lab
Department of HHPR
Rena Marrs McLean Gymnasium Room 120
254/710-4034
Sean Foster
sean_foster@baylor.edu

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REFERENCES

Blomstrand, E., Eliasson, J., Karlsson, H. K., & Kohnke, R. (2006). Branched-Chain Amino Acids Activate Key Enzymes in Protein Synthesis after Physical Exercise. *The Journal of Nutrition* , 269S-273S.

Bird, S., Tarpenning, K., & Marino, F. (2006). Independent and combined effects of liquid carbohydrate/essential amino acid ingestion on hormonal and muscular adaptations following resistance training in untrained men. *European Journal of Applied Physiology*, 225-238.

Bird, S., Tarpenning, K., & Marino, F. (2006). Liquid carbohydrate/essential amino acid Ingestion during a short-term bout of resistance exercise suppresses myofibrillar protein degradation. *Metabolism: Clinical and experimental*, 570-577.

Borsheim, E., Cree, M. G., Tipton, K. D., Elliot, T. A., Aarsland, A., & Wolfe, R. R. (2004). Effect of carbohydrate intake on net muscle protein synthesis during recovery from resistance exercise. *Journal of Applied Physiology*, 674-678.

Calders, P., Pannier, J.-L., Matthys, D. M., & Lacroix, E. M. (1997). Pre-exercise branched-chain amino acid administration increases endurance performance in rats. *Medicine & Science in Sports & Exercise* , 1182-1186.

Candeloro, N., Bertini, I., Melchiorri, G., & De Lorenzo, A. (1995). Effects of prolonged administration of branched-chain amino acids on body composition and physical fitness. *Minerva Endocrinologa* , 217-23.

Crowe, M. J., Weatherson, J., & Bowden, B. (2006). Effects of dietary leucine supplementation on exercise performance. *European Journal Of Applied Physiology* , 664-672.

Dreyer, H., Drummond, M., Pennings, b., Fujita, S., Glynn, E., Chinkes, D., et al. (2008). Leucine Enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR Signaling and protein synthesis in human muscle. *American Journal of Physiology. Enocrinology and Metabolism*, E392-400.

Drummond, M. J., Dreyer, H. C., Fry, C. S., Glynn, E. L., & Rasmussen, B. B. (2009). Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling. *Journal of Applied Physiology*, 1374-1384.

Feng, Z. (2010). p53 Regulation of the IGF-1/AKT/mTOR Pathways and the Endosomal Compartment. *Cold Spring Harbor Perspectives in Biology*.

Fujita, S., Dreyer, H., Drummond, M., Glynn, E., Volpi, E., & Rasmussen, B. (2009). Essential amino Acid and carbohydrate ingestion before resistance exercise does not enhance postexercise muscle protein synthesis. *Journal of Applied Physiology*, 1730-1739.

Gleeson, M. (2005). Interrelationship between Physical Activity and Branched-Chain Amino Acids. *The Journal of Nutrition* , 1591S-1595S.

Hong, S.-O. C., & Layman, D. K. (1984). Effects of Leucin on In Vitro Protein Synthesis and Degredation in Rat Skeletal Muscles. *The Journal of Nutrition* , 804-1212.

Karlsson, H. K., Nilsson, P.-A., Nilsson, J., Chibalin, A. V., Zierath, J. R., & Blomstrand, E. (2004). Branched-chain Amino Acids Increase P70S6K Phosphorylation in Human Skeletal Muscle After Resistance Exercise. *American Journal of Physiology: Endocrinology and Metabolism* , 1-7.

Kerksick, C. M., Rasmussen, C. J., Lancaster, S. L., Magu, B., Smith, P., Melton, C., et al. (2006). The Effects of Protein and Amino Acid Supplementation on Performance and Training Adaptations During Ten Weeks of Resistance Training. *Journal of Strength and Conditioning Research* , 643-653.

Kreider, R. B. (1999, March). Effects of Protein and Amino Acid Supplementation on Athletic Performance Retrieved September 02, 2010, from sportscience.org: http://www.sportsci.org/jour/9901/rbk.html

Liu, Z., Jahn, L. A., Long, W., Frybug, D. A., Wei, L., & Barrett, E. J. (2001). Branched Chain Amino Acids Activate Messanger Ribonucleic Acid Translation regulatory Proteins in Human Skeletal Muscle, and Glococorticoids Blunt This Action. *The Journal of Clinical Endocrinology and Metabolism* , 2136-2143.

Matsumoto, K., Koba, T., Hamada, K., & Mitsuzono, R. (2009). Branched-Chain Amino Acid Supplementation Increases the Lactate Threshold during and Incremental Exercise Test in Trained Individuals. *Journal of Nutritional Science Vitaminology* 52-58.

Mero, A. (1999). Leucine Supplementation and Itensive Training. *Sports Med* , 27(6): 347-58.

Mourier, A., Bigard, A., de Kerviler, E., Roger, B., Legrand, H., & Guezennec, C. (1997). Combined effects of caloric restriction and branched-chain amino acid supplementation on body composition and exercise performance in elite wrestlers. *International Journal of Sports Medicine* , 47-55.

Nair, K. S., & Short, K. R. (2005). Hormonal Signaling Role of Branched-Chain Amino Acids. *The Journal of Nutrition* , 1547S-1552S.

Nissen, S., Ray, S. M., Rathmacher, J., Rice, D., Fuller, J., Connelly, A., et al. (1996). Effect of Leucine Metabolite β-hydroxy-β-methylbutyrate. *Journal of Applied Physiology* , 2095-2104.

Rasmussen, B., Tipton, K., Miller, S., Wolf, S., & Wolfe, R. (2000). An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *Journal of Applied Physiololgy*, 386-392.

Rennie, M. J., Bohe, J., Smith, K., Wackerhage, H., & Grennhaff, P. (2006). Branched-Chain Amino Acids as Fuels and Anabolic Signals in Human Muscle. *The Journal of Nutrition* , 264s-268s.

Rieu, I., Balage, M., Sornet, C., Giraudet, C., Pujos, E., Grizard, J., et al. (2006). Leucine Supplementation Improves Muscle Protein Synthesis in Elderly Men Independently of Hyperaminoacidaemia. *Journal of Physiology* , 305-315.

Rieu, I., Sornet, C., Bayle, G., Prugnaud, J., Pouyet, C., Balage, M., et al. (2003). Leuicine-Supplemented Meal Feeding for Ten Das Beneficially Affects Postprandial Muscle Protein in Old Rats. *The Journal of Nutrition* , 1198-1205.

Sharp, C. P., & Pearson, D. R. (2010). Amino Acid Supplements and Recovery from High-Intensity Resistance Training. *Journal of Strength Conditioning Research* , 1125-1130.

Shimomura, Y., Murakami, T., Nakai, N., Nagasaki, M., & Harris, R. A. (2004). Exercise Promotes BCAA Catabolism: Effects of BCAA Supplementation on Skeletal Muscle during Exercise. *The Journal of Nutrition* , 1583s-1587s.

Shimomura, Y., Yamamoto, Y., Bajotto, G., Sato, J., Murakami, T., Shimomura, N., et al. (2006). Nutraceutical Effects of Branched-Chain Amino Acids on Skeletal Muscle. *The Journal of Nutrition* , 529s-532s.

Tom, A., & Nair, K. S. (2006). Assessment of Branched-Chain Amino Acid Satus and Potential for Biomarkers. *The Journal of Nutrition* , 324S-330S.

Waknine, Y. (2004). Amino Acids, Carbohydrate Supplementation Reduce Muscle Loss Due to Immobility, Bedrest. *Journal of Clinical and Endocrinological Metabolism*, 4351-4358.

Wilkinson, S. B., Phillips, S. M., Atherton, P. J., Patel, R., Yarasheki, K. E., Tarnopolsky, M. A., et al. (2008). Differential effects of resistance and endurance exercise in the fed state on signaling molecule . *Journal of Physiology*.

Yang, J., Chi, Y., Burkhardt, B. R., Guan, Y., & Wolf, B. A. (2010). Leucine Metabolism in Regulation of Insulin Secretion from Pancreatic Beta Cells. *Nutrition Reviews* , 270-279.