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Effects of Branched-Chain Amino Acid Supplementation on Exercise Induced Muscle Damage and Delayed Onset of Muscle Soreness after a Bout of Eccentric Exercise

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Abstract

Branched-chain amino acids (BCAA) have the potential to reduce both exercise-induced muscle damage (EIMD) and delayed onset of muscle soreness (DOMS) after a bout of eccentric contractions (ECC). The purpose of this study was to determine the effects of BCAA supplementation for 12 days prior to and for an additional 7 days following a bout of ECC on EIMD and DOMS. Sixteen women with no prior experience with strength training were assigned randomly to a BCAA group or a placebo group. Only four participants completed the trial due to COVID-19 (another completed 72h of recovery and was included). Participants supplemented their diet with either 400mg•kg^{-1} of BCAA or fibre (placebo) for 19 days. On day 13 each participant performed 3 sets of 15 repetitions of ECC and EIMD parameters were measured throughout a week of follow-up. A definitive conclusion is not possible given the limited data. However, our data suggest that this BCAA supplementation protocol can mitigate some effects of EIMD such as perceived muscle soreness but would fail to provide a full recovery of maximal force output. More study involving BCAA supplementation and exercise damage is required to assess how BCAA affects EIMD and DOMS.

Keywords

Protein supplementation, strength exercise, muscle soreness, hand-held dynamometer, ultrasound imaging

Summary for Lay Audience

Amino acids (AA) are the building blocks of body protein. The branched-chain amino acids (BCAA) are a group of three essential (cannot be made by the body) amino acids (leucine, isoleucine and valine) which are often recommended to boost muscle protein synthesis (MPS), especially leucine and/or enhance exercise performance. Further, some data suggest that BCAA supplementation can reduce exercise-induced muscle damage (EIMD) and muscle soreness after exercise. However, there is no consensus regarding the optimal amount of BCAA that an individual should consume to reduce EIMD and soreness. Therefore, the purpose of this experiment was to examine if 400mg•kg body mass^{-1} daily BCAA supplementation starting 12days prior to ECC exercise and throughout a week after exercise could mitigate muscle damage and soreness. The muscle damaging exercise involved 3 sets of 15 repetitions of ECC elbow muscle extension. 16 women with no strength training experience were assigned randomly into two groups (BCAA vs fibre). Only 4 completed all data collection due to COVID-19. Our data suggest that BCAA supplementation can reduce both EIMD and muscle soreness. More research is needed to establish the optimal dose for BCAA consumption for different types of exercise.

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COVID-19 and impact on research

In March 2020 the worldwide COVID-19 pandemic hit Canada, our lab was shut down, and our experiment had to stop. This has been very stressful for me as I planned to publish this work to demonstrate my competitiveness for PhD programs. However, I recognize that the health and safety of my participants, the lab volunteers, my roommates and myself is most important. As a result, rather than the traditional approach, this thesis focuses on methodology, preliminary data/expected results, as well as plans for future experiments.

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List of Abbreviations

AP	Acromion Process
AA	Amino Acids
BCAA	Branched-Chain Amino Acids
CSA	Cross-Sectional Area
CON	Concentric Contractions
CEAA	Conditionally Essential Amino Acids
DOMS	Delayed Onset of Muscle Soreness
DTI	Diffusion Tensor Imaging
ECC	Eccentric Contraction
EAA	Essential Amino Acids
EIMD	Exercise Induced Muscle Damage
HHD	Hand-Held Dynamometer
LE	Lateral Epicondyle
MVC	Maximal Voluntary Contraction
MRE	Magnetic Resonance Elastography
NEAA	Non-Essential Amino Acids
NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
PPD	Pain-Pressure Dynamometer
PAR-Q	Physical Activity Readiness Questionnaire
PGs	Prostaglandins
PLAC	Placebo
PPD	Pain Pressure Dynamometer
PPT	Pain Pressure Threshold

PZT	Lead Zirconate Titanate
ROM	Range of Motion
RBE	Repeated-Bout Effect
RM	Repetition Maximum
RPE	Rate of Perceived Exertion
RT	Resistance Training
SWE	Ultrasound Shear Wave Elastography
VAS	Visual Analogue Scale
VO_2max	Maximal Oxygen Consumption

Chapter 1

1 Introduction

Exercise-induced muscle damage (EIMD) is common among both elite and novice athletes, as well as individuals whose occupation involve eccentric or muscle lengthening contractions (ECC) (Fridén et al., 1986). On the other hand, concentric or muscle shortening contractions (CON) result in much less EIMD and delayed onset muscle soreness (DOMS). DOMS is the discomfort in muscles that occurs in the days following unaccustomed exercise and is associated with swelling and inflammation (Clarkson & Stephen, 1999), stiffness (Jones et al., 1997), reduced range of motion (Lee et al., 2002), and reduced force production (Clarkson et al., 1992; Warren et al., 2002). Typically, the discomfort peaks between 24 to 72 hours after the exercise bout and diminishes significantly after 5-7 days (Jones et al., 1986). Further, EIMD and DOMS cause both poor muscle performance and reduced mobility (Byrne & Eston, 2002; Cheung et al., 2003). As a result, it is important to develop strategies to mitigate these ECC effects on muscles.

Several dietary supplements are available to reduce EIMD. One popular example is branched-chain amino acids (BCAA). BCAA (leucine, isoleucine, and valine) are indispensable amino acids (AA) thought to diminish muscle protein degradation (Nair et al., 1992) as well as to stimulate muscle protein synthesis (MPS) (Garlick, 2005; Leenders & van Loon, 2011; Wolfe, 2017). However, previous studies have not been able to show consistent effects relative to muscle soreness and damage for several reasons. The major limitations include insufficient data to determine the effective dosage or necessary duration of BCAA supplementation, as well as poor quantification of any experimental muscle damage. However, based on a meta-analysis (Fouré & Bendahan, 2017), to be effective, the dose of BCAA supplementation should be at least 200mg•kg body mass^{-1}•day^{-1} for at a minimum of 10 days prior to muscle damaging exercise. Our study was designed using the above suggestions.

Chapter 2

2 Literature Review

2.1 Amino Acids (AA)

AA are organic substances that contain an amino group and an acid group (Wu, 2009). All AA have four different atoms or groups binding to the carbon backbone except for glycine, as it does not have a R group (sidechain) unlike other AA. Only 20 out of the several hundred naturally occurring AA serve as the building blocks of protein (Jalkanen et al., 2003), but some non-protein AA such as taurine are also important in cell metabolism (Hayes et al., 1975). AA also serve as critical regulators of a great many cellular processes (Figure 1) including growth (Meisinger et al., 1964), immunity (Li et al., 2007) and reproduction (Hemmings et al., 2013).

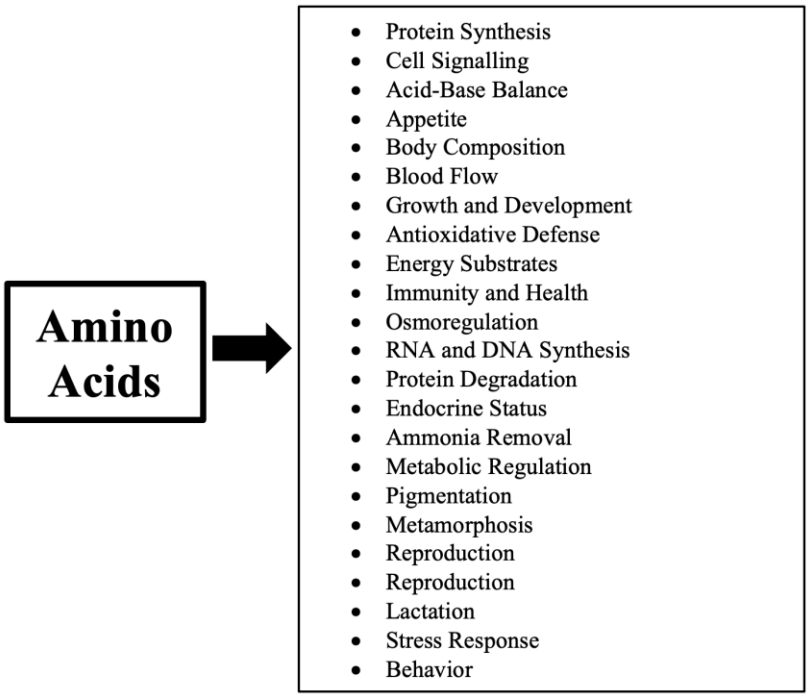

Figure 1. Regulatory functions and roles of AA on whole-body homeostasis (Adapted from Wu, 2010).

AA are categorized as essential amino acids (EAA), conditionally essential amino acids (CEAA) and non-essential amino acids (NEAA). EAA and NEAA are also known as indispensable and dispensable AA, respectively (Reeds, 2000). Indispensable AA must be in one's diet as the body is unable to make them in sufficient quantities for optimal body functioning. Conditionally indispensable AA can become indispensable under certain stressful conditions, including prolonged exercise (Walsh et al., 1998).

Typically, CEAA can be synthesized in amounts required for daily body need. However, stress can increase the amount of CEAA required and when this occurs dietary intake becomes critical (Castellanos et al., 2006; Wu, 2009). For example, proline is an EAA for burn patients (Jaksic et al., 1991) and glutamine is considered as an EAA in infants suffering from Duchenne muscular dystrophy (Hankard et al., 1999). Therefore, CEAA are usually considered as NEAA and the demand of CEAA is based on the physiological response of an individual. Typically, NEAA can be synthesized de novo by the body in adequate amounts for optimal health (Hou et al., 2015; Hou et al., 2017).

Table 1. Types of essential amino acids (EAA), conditionally essential amino acids (CEAA) and non-essential amino acids (NEAA) in healthy adults (Table adapted from Institute of Medicine U.S., 2005).

Essential	**Conditionally Essential**	**Non-Essential**
Histidine	Arginine	Alanine
Isoleucine	Cysteine	Aspartic acid
Leucine	Glutamine	Asparagine
Lysine	Glycine	Arginine
Methionine	Proline	Cysteine
Phenylalanine	Tyrosine	Glutamic acid
Threonine		Glutamine
Tryptophan		Glycine
Valine		Proline
		Serine
		Tyrosine

Importantly, the α-amino group of a number of amino acids can be transferred (transaminated) to the α-carbon of several α-ketoacids making a different amino acid (Braunstein & Kritzmann, 1937). In 1966, two groups of researchers (Ichihara & Koyama, 1966; Taylor & Jenkins, 1966) reported independently that the transamination of leucine, valine and isoleucine were all catalyzed through one single enzyme, (BCAA α-ketoglutarate transaminase). This specific enzyme is responsible primarily for catalyzing BCAA transamination, although there are still some reactions with norleucine and norvaline as well (Ichihara & Koyama, 1966).

Unfortunately, there are a number of limitations for transamination to synthesize enough EAA for our daily bodily functions (Reeds, 2000; Wu, 2009). First, keto-acids are not a part of our usual diets and, therefore, will not be 'ordinary available' to us (Reeds, 2000). This lack of precursor α-keto acids in our diet will in turn lead to insufficient EAA formation as a product of transamination. Consequently, EAA cannot be produced in sufficient amounts through transamination. Second, there are rate limiting steps in AA synthesis, including limited precursor AA, to act as donors of the accessory group or the carbon (Womack & Rose, 1947). As a result, as mentioned, it is not possible to generate enough EAA for optimal body function. Therefore, EAA must be consumed daily or health will be compromised (Ha & Zemel, 2003).

2.2 BCAA Catabolism

Unlike most amino acids, BCAA are not catabolized in the liver but rather in extrahepatic tissues, primarily in skeletal muscle (Holeček, 2018; Nie et al., 2018). This is due to the greater activity of branched-chain amino acid aminotransferase inside skeletal muscles, responsible for the first reaction in BCAA catabolism (Hall et al., 1993). This reaction is reversible and reverses the amino group of each BCAA to α-ketoglutarate (α-KG), forming their respective branched-chain keto acids (BCKA) (Figure 2) - α-ketoisocaproate (ketoleucine, KIC - ketogenic), α-keto-β-methylva-lerate (ketoisoleucine,

KMV - glucogenic), and α-ketoisovalerate (ketovaline, KIV – both ketogenic and glucogenic) as well as glutamate (Hutson et al., 1988).

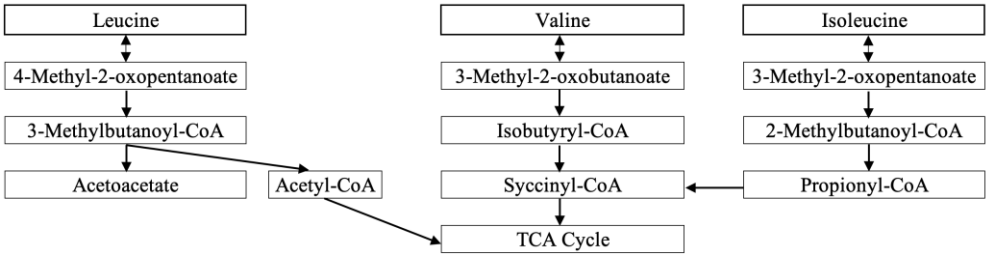

Figure 2. Pathways of BCAA catabolism (Adapted from Nie et al., 2018).

The branched-chain α-keto acid dehydrogenase complex (BCKDC), the second enzyme involved in BCAA catabolism, is a multienzyme complex that is regulated through phosphorylation-dephosphorylation (Hutson et al., 2005). It is located in the inner mitochondrial membrane of most cells (Pettit et al., 1978) and catalyzes the irreversible BCKA decarboxylation reaction to their respective branched-chain acyl-CoA esters (Harper et al., 1984). The further catabolism of KIC with BCKA produces acetyl-CoA and acetoacetate, KIV to succinyl-CoA and KMV to propionyl-CoA and acetyl-CoA (Holeček, 2018).

BCKD activity is low in the brain, adipose tissues and skeletal muscles, moderate in the heart and kidneys and highest in the liver (Harper et al., 1984). Despite this, skeletal muscles account for 35-40% of the total body mass (Janssen et al., 2000), so the overall utilization of BCAA in the skeletal muscles is significant. As a result, the major organs that catabolize BCAA are the liver and skeletal muscles (Figure 3) (Holeček, 2018).

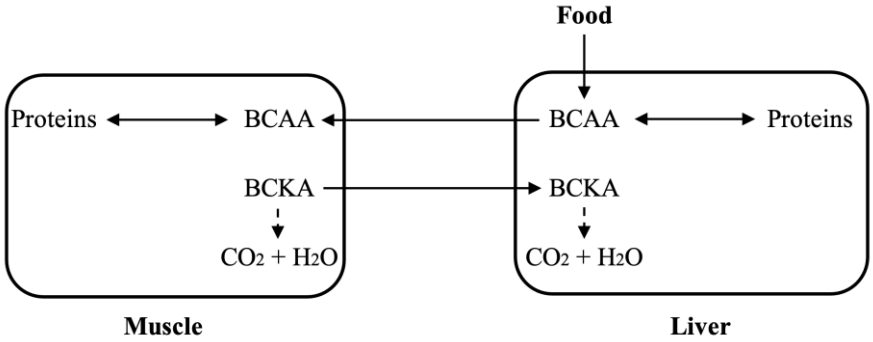

Figure 3. Liver and skeletal muscles are the major tissues responsible for BCAA catabolism (Adapted from Holeček, 2018).

2.2.1 Eccentric (ECC) and Concentric (CON) Contractions

Regular strength exercise leads to muscle hypertrophy, due to an increase in muscle fibre size not number (Goldberg et al., 1975). Although the best training regime is still not known (Mitchell et al., 2012; Phillips, 2009), it is common for athletes to perform 3-5 sets of 6-12 maximum repetitions (RM) according to the recommendations from American College of Sports Medicine (ACSM, 2009). Both ECC (lengthening) and CON (shortening) contractions occur when performing routine strength training or daily activities of living. Muscles produce force through the interaction of the contractile filaments, actin and myosin after cross-bridge interaction. When the maximum number of myosin filaments overlap with the maximum number of actin filaments at the optimum sarcomere length, the muscle generates maximum force (Gordon et al., 1966; Huxley & Simmons, 1971). In addition, muscle force varies with the velocity of lengthening (Katz, 1939) and shortening (Hill, 1938).

When the force exerted exceeds the load to be lifted muscle shortening occurs and the load is raised. If the exerting force is greater than the force that the muscle can generate, lengthening contractions will occur due to gravity. The mechanisms behind these two types of muscle contraction differ and ECC is able to generate more force compared to CON (Levin & Wyman 1927). In fact, ECC can generate ~ twice the amount of muscle force when compared to CON contractions (Ebbeling & Clarkson, 1989). There are theories that suggests during ECC, cross-bridges do not detach and reattach as frequently as CON, and thus do not require as much ATP (Huxley, 1975). Hence, ECC is known for ‘low cost and high force’ when compared to CON and even isometric contraction (Ortega et al., 2015). Moreover, ECC are prone to greater muscle damage vs CON or isometric (static) contractions (Hunter & Faulkner, 1997).

2.3 Exercise-Induced Muscle Damage (EIMD)

EIMD is much greater after bouts of ECC vs CON (Fridén et al., 1986) and is characterized by myocellular enzymes and proteins efflux (e.g., myoglobin, creatine

kinase, etc.), reduced range of motion (ROM), edema, loss of muscle strength, myofibrillar disruption and/or delayed onset of muscle soreness (DOMS) (Hyldahl & Hubal, 2014). Enzyme efflux appears to be the result of a secondary muscle membrane damage because of inflammation (Howatson & van Someren, 2008).

Mechanical tension caused during exercise can also disturb the integrity of skeletal muscles (Schoenfeld, 2012). During ECC, ~ half the number of sarcomeres will overstretch beyond the length of overlapping and become nonuniform, causing "popped sarcomeres" and muscle force reduction (Hyldahl & Hubal, 2014; Morgan & Proske, 2004). The sarcolemma and the t-tubule structure will be overloaded with an efflux of calcium ions (Al-Qusairi & Laporte, 2011; Byrd, 1992; Duan et al., 1990). This will lead to the dysfunction in the coupling of the excitation-contraction, membrane disruption and the opening of the stretch-activated channels (Duan et al., 1990; Hyldahl & Hubal, 2014). Interestingly, some believe that the increased muscle protein turnover and inflammation associated with EIMD is necessary for muscle hypertrophy (Evans & Cannon, 1991) while some suggest the opposite (Grant, et al., 2015; Nosaka et al., 2003; Zeviani, 2008). This will be discussed further in section 2.5. In addition, only ECC causes an elevation of the intramuscular pressure in the anterior compartments of the exercised muscles and this could be one of the causes for DOMS (Faulkner & Brooks, 1993). The recovery process of EIMD can take up to 72 hours (Barnett, 2006; Nedelec et al., 2013). Of course, a faster recovery could be beneficial as it would enable athletes to engage in strength training more frequently (Howatson & van Someren, 2008).

2.4 Delayed Onset of Muscle Soreness (DOMS)

Many athletes experience DOMS at some point of their training or activity, especially after unaccustomed muscle contractions involving repeated bouts of eccentric contractions (Cheung et al., 2003). Mainly, the discomfort comes from the skeletal muscles and increases during the first 24 hours after exercise. Often the peak occurs between 24 to 72 hours and then diminishes gradually, usually disappearing by 5-7 days

(Jones et al., 1986). Typically, the discomfort is associated with muscle inflammation and edema (Clarkson & Stephen, 1999), altered motor control (Carson et al., 2002; Slater et al., 2003), reduced range of motion (Lee et al., 2002), muscle stiffness (Jones et al., 1997) and reduced force production (Clarkson et al., 1992; Warren et al., 2002). There are at least six hypothesized mechanisms to explain DOMS – enzyme efflux, inflammation, muscle damage, connective tissue damage, muscle spasm and lactic acid accumulation (Armstrong, 1984). Likely DOMS involves a combination of several of these factors (Cheung et al., 2003).

Although the pathology of DOMS is often subclinical (Cheung et al., 2003), it is very common and the level of discomfort can vary from slight to a severe pain that can limit movement significantly. Being able to reduce DOMS can be beneficial to anyone who performs eccentric contractions due to work or training requirements.

2.5 BCAA supplementation and EIMD

Over the years, ‘no pain no gain’ has become a popular phrase among strength trainers. In fact, many believe that muscle soreness after an unaccustomed bout of exercise is necessary for adaptation including muscle hypertrophy and strength gains (Evans & Cannon, 1991). On the other hand, a study by Flann and colleagues (2011) indicates that muscle rebuilding is independent of EIMD. They divided participants into two groups, a pre-trained (PT) group that performed 11 weeks of ECC resistance training (RT) and the naïve (NA) group performed 8 weeks of ECC RT. The authors proposed that participants from the PT group would experience less muscle damage as there was an acclimation to the ECC RT, whereas the NA group would experience more severe muscle damage. However, after the completion of RT, both groups gained the same net muscle strength and size, suggesting that total work done is the critical factor that affects muscle adaptation, rather than EIMD and DOMS. Therefore, it follows that a reduction of EIMD with BCAA would not lead to a reduction in muscle adaptation. Further, studies have also shown that muscle adaptation can occur with strength exercise regardless the degree of

EIMD (Flann et al., 2011; Foley et al., 1995). Other review articles concur that muscle soreness is not necessary to attain muscle adaptations so the belief of 'no pain no gain' in RT appears to be incorrect (Grant, et al., 2015; Nosaka et al., 2003; Zeviani, 2008). On the other hand, previous literature has demonstrated that chronic protein supplementation can lead to positive effects on muscle power, strength and mass (Pasiakos et al., 2015). Moreover, it appears that the current daily protein recommendation guidelines, $0.8g•kg^{-1}$ (Institute of Medicine, 2005) are insufficient for athletes. For example, daily recommendations based on nitrogen balance data are as high as $2.0g•kg^{-1}$ (Thomas et al, 2016) for strength-trained athletes and 1.2 - $1.6g•kg^{-1}$ for endurance athletes (Friedman & Houltham & Rowlands, 2014; Lemon, 1989; Lemon et al., 1997). Further, recent indicator AA oxidation data confirm these elevated protein intake recommendations (Bandegan et al., 2017; Bandegan et al., 2019).

Similarly, there is controversy regarding the optimal amount of BCAA supplementation due to variable results shown in the current literature. There are studies that show no benefits of BCAA supplementation (Areces et al., 2014; Knechtle et al., 2012) as well as others reporting reduced EIMD and related symptoms (DOMS) (Fouré et al., 2016; Howatson et al., 2012; Shimomura et al., 2006; Shimomura et al., 2014). Unfortunately, no standard dose of BCAA consumption has been established to minimize the effects of EIMD and whether BCAA should be supplemented chronically or acutely is still not clear. Further, the intensity, frequency, duration and type of exercise also has an impact on the effectiveness of BCAA supplementation and these factors vary widely in the literature. A systematic review done by Fouré and Bendahan (2017) concluded that positive outcomes of BCAA supplementation on EIMD are possible when >$0.2g•kg\ body\ mass^{-1}•day^{-1}$ of BCAA are consumed >10 days prior to the muscle damaging protocol whenever the extent of muscle damage is low to moderate. For example, a study done by Coombes and McNaughton (2000) reported that 14 days of $12g•day^{-1}$ of BCAA supplementation ($0.17\ g•kg^{-1}$ for a 70 kg mass) reduced EIMD markers (serum creatine and lactate dehydrogenase) of healthy men after cycling at 70% of their maximal oxygen consumption (VO_2 max) for 120 minutes. Another study (Howatson et al., 2012) found that a daily dosage of 20g BCAA supplementation for 12 days and a 20g BCAA bolus

prior to and immediately after the damaging protocol reduced EIMD in healthy males after 100 consecutive drop-jumps.

There are two possible physiological mechanisms to explain how BCAA supplementation could mitigate muscle soreness and damage. First, by scavenging reactive oxygen species (Valerio et al., 2011) and second, by reducing muscle-protein breakdown (Fouré & Bendahan, 2017; MacLean et al., 1994). Specifically, it has been suggested that leucine can reduce protein oxidation after ECC exercise (Shimomura et al., 2009), suppress muscle proteolysis (Zanchi et al., 2008), and reduce symptoms of muscle damage (da Luz et al., 2011). Moreover, both chronic (Sharp & Pearson, 2010) and short-term BCAA supplementation (Kirby et al., 2012; Shimomura et al., 2010) reduce post ECC exercise serum creatine kinase (CK), a biomarker of muscle damage. However, as dosage and supplementation regimes vary widely more studies are needed to establish a definitive BCAA supplementation protocol.

2.6 Image-Based Methods to Measure EIMD/DOMS

Biomarkers in blood plasma such as myoglobin and creatine kinase (CK) are standard indicators for muscle damage (Byrnes et al., 1985; Clarkson et al., 1988; Hansen et al., 1982; Newham et al., 1987; Nosaka et al., 1991; Paul et al, 1989). In addition, structural damage (z-line streaming), often called myofibrillar disruption has been reported in muscle needle biopsy samples (Staron et al., 1992). Although both methods provide valuable information, each has limitations. For instance, enzyme efflux is inconsistent relative to damage and pain sensation and muscle biopsies are somewhat invasive. Hence, imaging and performance measures are more viable methods to access muscle damage. Ultrasound based muscle cross-sectional area (CSA) and echo intensity (Damas et al., 2016), magnetic resonance elastography (MRE), ultrasound shear wave elastography (SWE), diffusion tensor imaging (DTI) and dynamic imaging are some of the possible non-invasive image-based methods that can assess properties and show alterations of the skeletal muscles (Bilston et al., 2019). Nevertheless, when compared to MRI and the

other imaging technologies, ultrasound is less expensive, as well as more accessible and versatile. For these reasons, ultrasound imaging is a useful option to access muscle damage (Damas et al., 2016).

2.6.1 Ultrasound – Basic Physics

Ultrasound involves mechanical or longitudinal compression (sound) waves with a frequency higher than 20kHz (McGahan & Goldberg, 1998). Importantly, these waves can propagate through different materials, causing vibration of particles that are transverse or parallel to the direction of propagation of the sound waves. Longitudinal waves have the ability to propagate through all materials, but only transverse sound waves can propagate through solids (Dance et al., 2014). The highest speed occurs when travelling through solids, intermediate in liquids and lowest when in gases (Lacefield, 2014). Velocity, wavelength, frequency, pressure, power and intensity are the different parameters of an ultrasound wave.

Acoustic energy from the beam will attenuate due to absorption, deflection (including refraction, reflection and scattering (see Figure 4), and divergence as the wave penetrates through the body. Reflection of the wave is known as an echo, where acoustic impedance of different materials and the orientation of the beam and reflecting surface can affect the magnitude of the echo (Ziskin, 1993). In contrast, refraction is the deviated ultrasound beam when the wave is propagating through the boundary between two different material types. Acoustic impedance is the product of propagation speed and density (Aldrich, 2007). Attenuation (including absorption, deflection and divergence) is the loss of energy per distance - as sound waves travels along a material, it will have to overcome internal friction of the material, this will lead to decrease in energy (Ziskin, 1993).

Table 2. Variables of ultrasound

Velocity (v)	Rate of sound energy propagation in a specific direction; mainly determined by the characteristics of the materials, such as stiffness and density (Aldrich, 2007). When the direction of propagation is not specified, speed should be used instead of velocity as velocity is a vector quantity and the magnitude has to be specified (Ziskin, 1993). Velocity (v) = Wavelength (λ) × Frequency (f)
Wavelength (λ)	It is the distance between two amplitudes of a wave.
Frequency (f)	Number of complete oscillations, pressure changes or number of cycles that occurs to each particle per second (Abu-Zidan, Hefny & Corr, 2011; Aldrich, 2007; Ziskin, 1993).
Power (watts)	One of the two measures for the strength of the ultrasound wave. Without attenuation, power of an ultrasound wave will remain constant and it is the total amount of energy required from an acoustic wave to pass through a material per time period (Ziskin, 1993).
Intensity (W/m^2)	One of the two measures for the strength of the ultrasound wave. It is the transfer of energy per unit cross-sectional area. Intensity is dependent on the width of the beam, a narrow and focused beam will have a higher intensity than wide and dispersed beam (Ziskin, 1993).

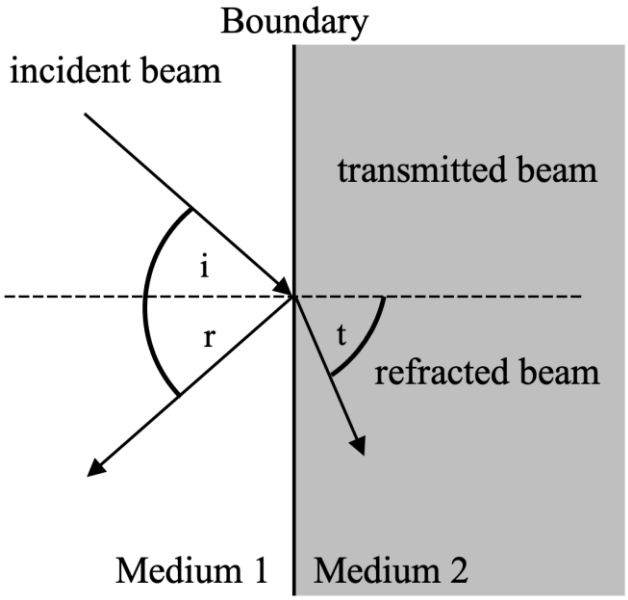

Figure 4. Ultrasound refraction and reflection (Adapted from Aldrich, 2007).

2.6.2 Ultrasound – Clinical Physics

The ultrasound frequency used in clinical settings ranges between 2 to 15MHz (Hangiandreou, 2003). Soft tissues are seen as liquids in the acoustical point of view, thus longitudinal waves can propagate through soft tissues. On the other hand, compact bones are the only type of tissue that transverse sound waves can propagate through (Ziskin, 1993). Ultrasound waves and their reflected echoes are received by medical ultrasound machines (Abu-Zidan, Hefny & Corr, 2011) and the basic mode that is used most often is known as the brightness mode (B mode) (Hangiandreou, 2003). B mode images are black and white depending on the area where the image was taken, and the images are two dimensional (2D). Depending on the position of the ultrasound probe, images can represent oblique, transverse, coronal and sagittal planes of the human body (Hangiandreou, 2003).

Modern ultrasound transducers are made up of ferroelectric ceramic lead zirconate titanate (PZT) and a non-piezoelectric polymer. By inserting a piezoelectric crystal between the two, sensitivity and bandwidth of the transducer can be improved (Lacefield,

2014). Piezoelectricity was first discovered in quartz by Jacques and Pierre Curie in 1880. Piezoelectric crystals change its arrangements and cause deformation in the crystal when a difference in voltage was applied. On the other hand, when an external force is applied and deforms the crystal, the dipoles will rearrange and induce a net charge across the crystal (Lacefield, 2014). These properties can be useful to an ultrasound probe as it can transfer electrical signals to mechanical vibrations and vice versa (Hangiandreou, 2003), thus transmitting a focused pulse along a specified line of sight, which is the scan line (Lacefield, 2014).

When the ultrasound wave passes through tissues in the human body, the wave will be reflected back, picked up by the transducer and shown as an image on the screen (John, 1997). Reflective waves are stronger when the wave passes through a more solid material as the density of the material is greater (John & Aaron, 2006). Compared to solids, fluids are less dense and will attenuate more energy. Less ultrasound waves will be reflected back to the probe, producing an echogenic image (black). Reflective waves in solids like skeletal bones will generate bright images (white) with a black acoustic shadow known as the anechoic shadow behind the bone as ultrasound waves cannot pass through the solid (Andreas, 2008).

2.6.3 Ultrasound and Skeletal Muscles

Skeletal muscles on ultrasound images can be easily distinguished from their surrounding structures such as blood vessels, nerves, subcutaneous fat and skeletal bones (Peetrons, 2002). Muscles have a low echo intensity and are relatively black on the image as it is not very reflective, while the perimysial connective tissue is more reflective and the epimysium is highly reflective (Pillen & van Alfen, 2011). When the ultrasound probe aligns with the long axis of the muscle, the perimysial connective tissue will reflect the waves, generating an image with a triangular, linear or pinnate structure. When the ultrasound probe is perpendicular to the long axis of the muscle, perimysial connective tissue will also reflect the waves but in a speckled appearance (Pillen & van Alfen, 2011).

2.6.4 Ultrasound and EIMD

An increase in echo intensity (brightness) after muscle damage is found along with the reduction in MVC, decreased ROM, increased limb circumference (Nosaka & Sakamoto, 2001) increased muscle soreness, muscle component efflux (creatine kinase, myoglobin, and lactate dehydrogenase) (Chen & Nosaka, 2006; Gonzalez-Izal et al., 2014), increased pennation angle (Yu et al., 2015) and DOMS (Agten et al., 2017; Longo et al., 2016). Further, edema within the muscle belly appears as increased echogenicity (brightness) within the damaged muscle even without muscle architecture disruption (Bencardino et al., 2000).

2.7 Purpose and hypotheses

The purpose of this study was to determine the effects of BCAA supplementation for 12 days prior to and for an additional 7 days following a bout of ECC on EIMD and DOMS. It was hypothesized that BCAA supplementation can reduce EIMD characteristics, including 1) lower ROM, limb girth, myoglobin and ultrasound echogenicity; 2) higher MVC, pain pressure threshold (PPT) and VAS when compared to PLAC.

Chapter 3

3 Methods

3.1 Participants

Sixteen untrained women (no strength training in the previous 6 months) volunteered for this experiment but due to Covid-19 only 4 completed all data collection (Table 3). Another completed all data collection up to 72 hours post exercise. Each completed a medical screening questionnaire – Physical Activity Readiness Questionnaire (PAR-Q) (Bredin et al., 2013, Appendix A) and written informed consent was obtained before any participation (Appendix B). This study was approved by The Western University Health Science Research Ethics Board (HSREB) (Appendix C). All testing was completed in the Exercise Nutrition Research Laboratory at Western University.

3.2 Experimental Overview

Each participant was assigned randomly to one of two treatments (400mg BCAA•kg^{-1}•day^{-1} [BCAA400] or placebo (fibre supplement; PLAC). Supplements were provided in a single blind fashion, divided into 3 portions and ingested 1 hour after each daily meal for 19 days. Anthropometric measurements including body height, mass and composition (BodPod®; Life Measurements, Concord, California, USA) were collected prior to the study. Markers of muscle damage and soreness (see below for details) were collected before and for 7 days following an eccentric exercise bout (elbow extension) completed on day 1 of the menstrual cycle (early follicular phase) to standardize the experiment according to their menstrual cycle (Figure 5).

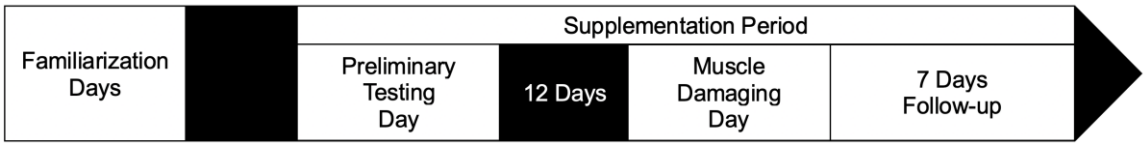

Figure 5: General overview of the experiment.

3.3 Familiarization Days

To minimize learning effects, all participants were familiarized to all measurements prior to the preliminary testing. This involved an opportunity to try out each procedure several times. MVC and PPT were all performed three times with the non-dominant arm and the mean of the last two was recorded. To familiarize participants to the rate of elbow extension to be used for inducing muscle damage, each flexed their elbow fully and practiced extending their arms approximately 30 degrees per second, reaching full elbow extension over 4 seconds. Participants completed the familiarization eccentric exercise (3-4 times). No external load was used in order to prevent muscle damage.

3.4 Preliminary Testing Day

Twelve days prior to the experiment, participants came into the lab in the morning (0700-0800h) following an overnight fast, with minimal physical activity (received a ride to the lab and use of the building elevator) and used the washroom. Participants then rested supine for 10 min and a series of measures were taken, including body temperature (ear canal), height, body mass, body composition, arm limb girth, ROM, arm ultrasound images, PPT, MVC (dominant arm), RPE, perceived soreness (VAS), blood sampling, and the Understand Pain Intake Questionnaire Package (Appendix D) (Figure 6).

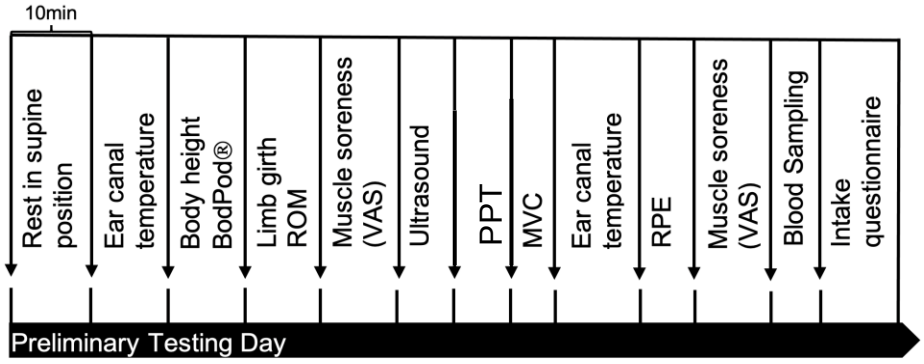

Figure 6: Preliminary testing day timeline. Range of motion (ROM), visual analog scale (VAS), pain pressure threshold (PPT), maximum voluntary contraction (MVC), rate of perceived exertion (RPE).

3.5 Diet and Activity Before Testing

A 3-day diary of all food and drink intake (2-weekdays and 1-weekend day) was recorded using the app MyFitnessPal® before the dietary supplementation started. Any participants with atypical diets were excluded. Participants were asked to refrain from any type of exercise at least 5 days prior to starting as well as and throughout the whole experiment.

3.6 Study Day Protocol

3.6.1 Prior to the Muscle Damaging Protocol

Participants came into the lab in the morning (0700-0800h) following an overnight fast, with minimal physical activity (received a ride to the lab and use of the building elevator) and used the washroom. Participants then rested supine for 10 min. Then, body temperature and body composition were measured. Next ROM, limb girth, perceived soreness (VAS) were assessed and ultrasound images of the biceps and brachialis on the dominant arm were taken. Finally, muscle pain and grip strength were measured and a standard, carbohydrate (CHO) snack, consisting of 7kcal•kg^{-1} jelly (Jello, Kraft Canada, Ontario, Canada) was provided. When all these measures were completed the participant performed a standardized warm-up procedure using free weights (2 sets of 10 repetitions with ~10% of 1RM; CON) before performing the experimental muscle damaging exercise bout (elbow eccentric exercise).

3.6.2 Muscle Damaging Protocol

The muscle damaging exercise bout involved elbow eccentric contractions starting from a position where the elbow was at full flexion. Specifically, while seated, the participants completed 3 sets of 15 repetitions with the dominant arm (3-minute rests between sets) at 70% of their isometric 1RM (Sakamoto et al., 2010). A spotter stood in front of each participant and prepared to catch the dumbbell just in case participants were not able to

resist the load. A metronome was utilized so that participants were able to extend their elbow fully over 4 seconds (extending approximately 30 degrees per second). Basically, participants resisted the downward movement of the load as best they could until full elbow extension was reached.

3.6.3 Muscle Damaging Protocol Post Measures

RPE, perceived soreness (VAS) and body temperature were measured immediately following the damaging protocol followed by a series of measures including limb girth, ROM, PPT, MVC, dominant arm ultrasound images and blood samples from the non-dominant arm (see below for details). Finally, participants completed the Understanding Pain Follow-Up Questionnaire package (Appendix E) (Figure 7).

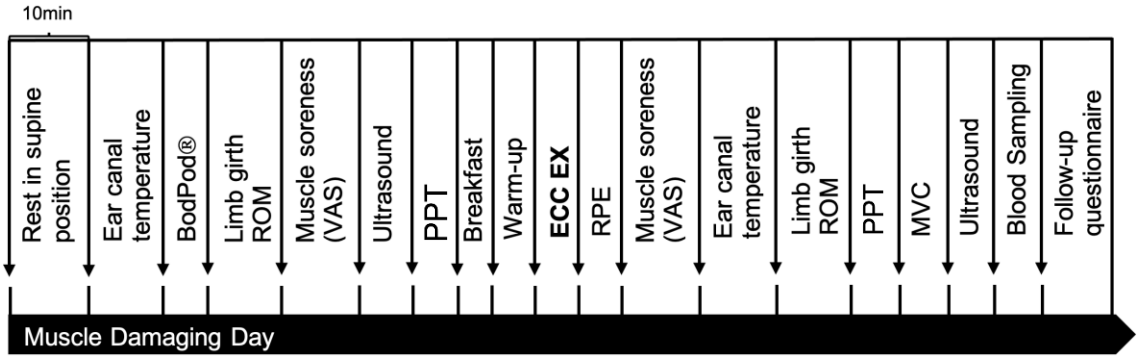

Figure 7: Muscle damaging day timeline. Range of motion (ROM), visual analog scale (VAS), pain pressure threshold (PPT), eccentric exercise (ECC EX), maximum voluntary contraction (MVC), rate of perceived exertion (RPE).

3.7 Follow-up Days Protocol

The participants returned to the lab and all measurements were taken as before at 24, 48, 72, 96, 120, 144 and 168 hours after the muscle damaging experimental exercise bout. Also, data on body composition and the Understanding Pain Follow-Up Questionnaire package (Appendix E) were collected on the last day of the follow-up (Figure 8).

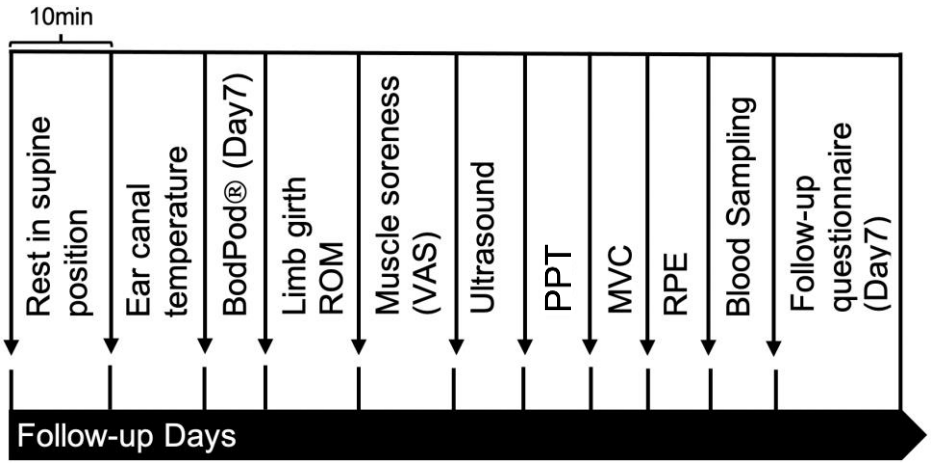

Figure 8: Follow-up days' timeline. Range of motion (ROM), visual analog scale (VAS), pain pressure threshold (PPT), maximum voluntary contraction (MVC), rate of perceived exertion (RPE).

3.8 Measurements

3.8.1 Anthropometric Measurements

A double ruler body scale (Health o meter®) was used to measure body height prior to the body composition measurement. Body density (body mass/body volume) was determined with the Bodpod®. Participants refrained from consuming food and drink for 2 hours prior to the measurements, used the washroom, if necessary and wore a sports-bra, compression shorts and a tight swimming cap on their head. These were done to ensure

minimal air was left under the clothing or between hair follicles, which can lead to inaccurate body volume measurements. A predictive equation was used to estimate thoracic gas volume of the participants and percent body fat data were calculated by the Bodpod® software using the Siri equation (Noreen & Lemon, 2006).

3.8.2 BCAA Supplement and Placebo

The BCAA supplement (Now Foods, Illinois, USA) contained ~2.6g of BCAA (1150mg of free-form leucine, 550mg of free-form isoleucine and 550mg of free-form valine) per level teaspoon. This provided a 1:0.5:0.5 ratio of leucine to isoleucine to valine similar to earlier studies (Areces, et al., 2014; Sharp & Pearson, 2010). The placebo (PLAC) group received fibre supplements (Walmart Equate Sugar Free Fiber Supplement Powder, Arkansas, USA). Both supplements were dissolved into Crystal Light liquid drink mix (Kraft Ontario, Canada) with cold water (250 ml). This zero-calorie drink mix was used to mask the taste of BCAA and the PLAC.

The amount of supplement given depended on the body mass of the participant (400mg BCAA•kg^{-1}•day^{-1}) divided into three doses and consumed one hour after each main meal. The supplements were provided in small plastic containers (one dose/container). Participants brought their supplement containers back every 3 days for refills and to verify compliance.

3.8.3 Supplementation Period

Participants provided information regarding their menstrual cycle (starting date of their menstrual period for the last two months and typical cycle duration). When the participants did not have a record of their previous period, they were instructed to inform the investigators once their next period began, a calculation was made to estimate the starting date of the subsequent menstrual cycle. Supplementation began 12 days before the estimated start of the menstrual cycle.

3.8.4 Range of Motion (ROM)

ROM was measured with a goniometer as the greatest angle of extension without pain as the participants were standing in the anatomical position. Participants were instructed to flex their elbow fully, then slowly extend their elbow and stop immediately when they felt discomfort. During the measurement, the lateral epicondyle was used as the reference point and one end of the goniometer was set perpendicular to the humerus.

3.8.5 Limb Girth

Arm limb girth was measured with an anthropometric tape midway between the elbow and shoulder as follows. Two anatomical landmarks – the acromion process (AP) and the lateral epicondyle (LE) were first located by palpating on the arm of each participant. An anthropometric tape was used to determine the midpoint between AP and LE. The midpoint was measured and marked with a felt marker when the arms were naturally hanging at the side when the participant was standing in the anatomical position.

3.8.6 Strength Measurement (MVC)

MVC was determined by three maximal repetitions of static elbow flexion (with each arm) using a handheld dynamometer (HHD; Lafayette Instrument, Indiana, USA) while seated on an incline bench. The HHD was anchored to a belt with Velcro, so when each participant pulled on the anchored HHD, the angle of the elbow was maintained at 90 degrees. The length of the belt was adjustable to accommodate participants with differing sitting heights. The length of the belt was recorded and used for each subsequent measure for that individual (Figure 9).

The belt was then looped around a tie down strap anchored to the floor. The bench was then positioned so the participants could sit on the bench with their back straight and their hip against the bench. Participants put their non-dominant arm across their chest, an

elastic Velcro strap was wrapped around their arm and shoulders to secure this position and minimize movement and possible effects caused by the non-dominant arm. The HHD was aligned with the styloid process and positioned one inch behind the wrist which was aligned with the corner of the bench. The elbow was not touching nor tucked into the trunk to minimize the possibility of pushing on the trunk (Figures 9 and 10).

A timer was started, and the participant pulled as hard as possible for 5s. Strong verbal encouragement was provided. After 5s, a 30s timer was started as a rest period and the HHD output was recorded. The above steps were repeated two more times. The mean of the last two measurements was taken as MVC.

Figure 9. MVC measurement using a handheld dynamometer (HHD) from in front.

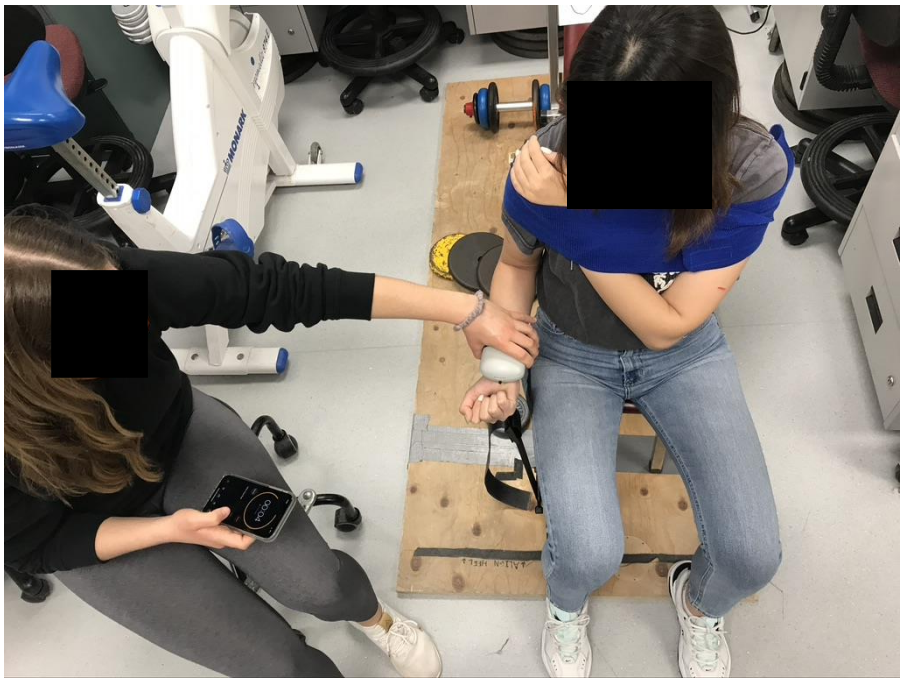

Figure 10. MVC measurement using a handheld dynamometer (HHD) from above.

3.8.7 Rate of Perceived Exertion (RPE)

RPE was measured immediately after all MVC measurements to assess the effort involved. Participants were presented with a Borg Scale (Borg, 1982) of 6 to 20 (6 means no exertion at all and 20 being extremely hard). They were asked to rate how hard the MVC exertion was and indicate the number that best represents their effort.

3.8.8 Pain Measurement

Using the mid-point of the arm (see 3.8.5 Limb Girth for details), the muscle belly of the biceps was located to assess the pain pressure threshold (PPT) in Newtons. Participants were asked to relax their muscles and rest both arms in their lap. The following standardized instructions were read to the participants: “I am going to start slowly applying pressure to the skin over top of your muscle. I want you to tell me the moment the sensation changes from pressure to pain”. The PPD was then positioned perpendicularly to the muscle belly, and pressure was applied by pushing against the muscle belly until the participant indicated pain. A 30s timer was started immediately as a resting period. This PPT measurement was repeated two more times following a 30 sec rest period. Then PPT measurements was repeated three more times with the elbow flexors contracted maximally. VAS was measured using a scale of 0-10 (1 being no pain and 10 being extremely painful).

Figure 11. PPT measurements using a pain pressure dynamometer (PPD).

3.8.9 Ultrasound Imaging

The ultrasound images of both the biceps and the brachialis were captured with an ultrasound machine (ATL Ultrasound Inc, APM Module, Philips Medical Systems,

Amsterdam, Netherlands) and the Epiphan software (version 3.29.1.0, Epiphan Video, Ottawa, Canada) with the participants lying supine on the massage table, and their muscles relaxed.

3.8.9.1 Biceps Brachii

Using the same location as the mid-point of the arm (see 3.8.5 Limb Girth for details), the muscle belly of the biceps was located. As outlined in the manual, ultrasound transmission gel (Aquasonic, Parker Laboratories Inc., Fairfield, New Jersey, USA) was squeezed onto a linear ultrasound transducer which was positioned parallel to the biceps for imaging purposes.

3.8.9.2 Brachialis Muscle

In order to locate the brachialis, the LE was palpated and an anthropometric tape was used to measure 7cm proximal from the LE and the imaging spot marked with felt marker (Nosaka & Sakamoto, 2001). The image was taken when the arms were naturally hanging at the side when the participant was standing in the anatomical position. The transducer was positioned perpendicular to the arm for imaging purposes.

3.8.10 Dietary Record

Participants completed a 3-day dietary record prior to the start of the experiment (2 weekdays and 1 weekend day). Briefly, they were asked to download a free phone application MyFitnessPal® (Under Armour, Baltimore, Maryland, USA) and input all food and liquid consumption to the app. They were asked to maintain their typical dietary habits throughout the experiment.

3.8.11 Muscle Soreness

Muscle soreness was measured using a visual analogue scale (VAS). Participants rated their perceived soreness at the moment on a scale of 1 to 10 (where 1 meant ‘no pain’ and 10 was ‘extremely painful) (Downie et al., 1978).

3.8.12 Biochemical Analysis

Blood samples were collected by venipuncture from an antecubital vein of the non-dominant arm into 8.5ml tubes with serum separator gel and clot activator. The vacutainers were immediately inverted gently 10 times, allowed to clot at room temperature for 30min and then centrifuged for 10 min (3000rpm) at 4ºC (Allegra 21R; Beckman Coulter, Mississauga, Ontario, Canada). Serum was then aliquoted and stored at -80degrees Celsius for later analysis.

3.8.13 Ultrasound Image Analysis

Ultrasound images were analyzed using ImageJ (Schneider, Rasband & Eliceiri, 2012) version 1.51 (U.S. National Institutes of Health, Bethesda, Maryland, USA) to quantify the echogenicity (grayscale) of the images in arbitrary units (echogenicity). To analyze the biceps images, a rectangle was selected to isolate the muscle area only, i.e., the fat layer and bone area were excluded (Figure 12). Similarly, for the brachialis analysis, an area of the brachialis, excluding fat and bone was outlined and assessed as shown in Figure 13.

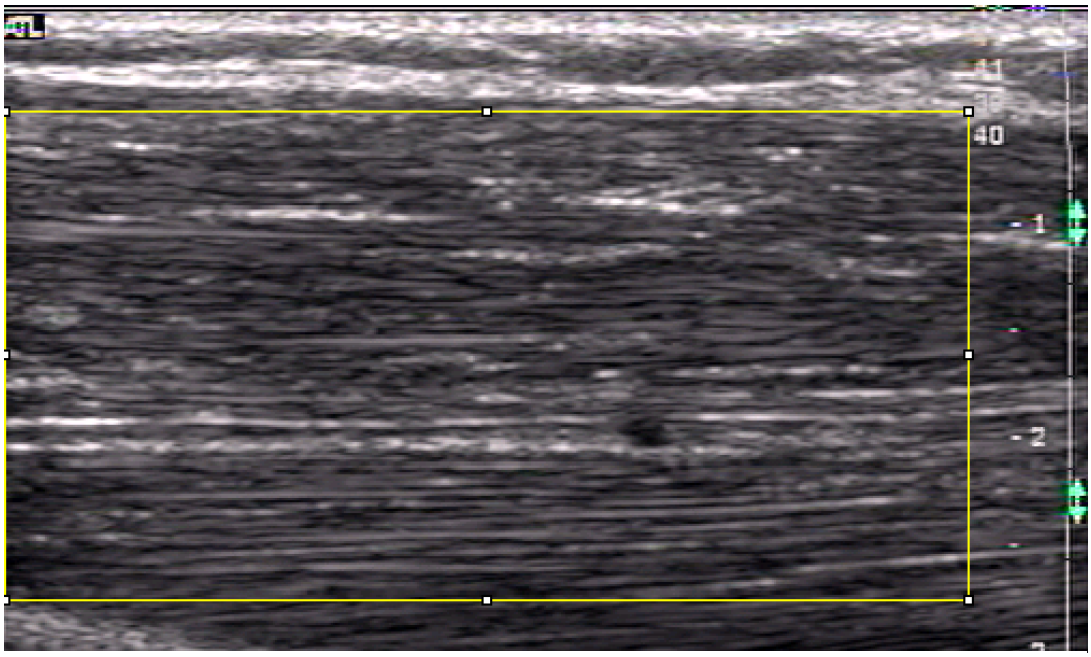

Figure 12: Biceps ultrasound image analysis.

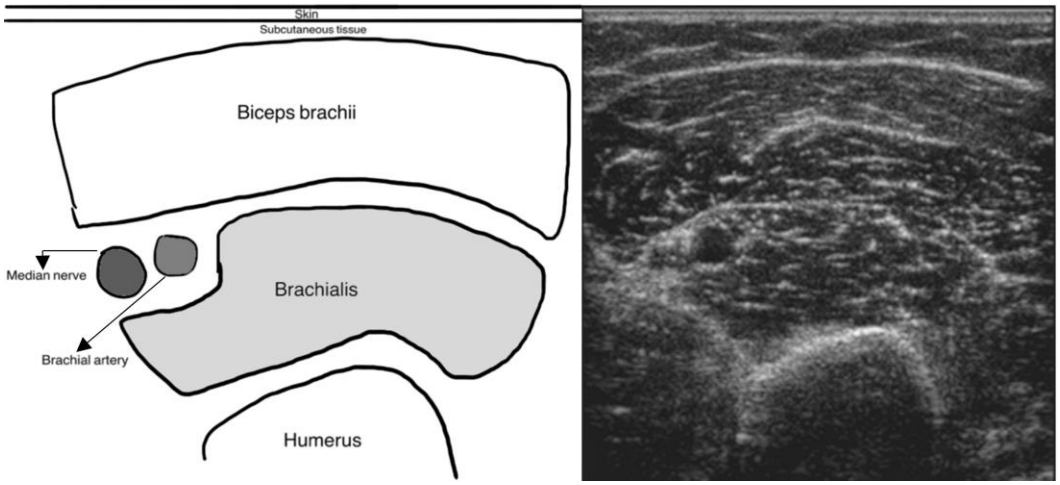

Figure 13: Brachialis ultrasound outline area (Adapted from Pillen, Arts and Zwarts, 2008).

3.8.14 Statistical Analysis

The plan was to use a two-way ANOVA (treatment X time) with post hoc testing where necessary using Sigmaplot version 12.5 (Systat Software Inc, San Jose, California, USA). Statistical analysis was not completed because of the same amount of data collected. Means ± SD are plotted.

Chapter 4

4 Result

4.1 Descriptive Analysis

16 female participants who did not have previous experience with strength training were recruited for this experiment. However, due to COVID-19, only four participants completed the whole study, three in the BCAA400 group and one in PLAC group. Further, two participants started the familiarization sections, two had completed the familiarization sections, one participant was in follow-up (day 3), three had completed the baseline measurements and four were about to start the experiment. Figures presented below include data from the four participants in BCAA400 (three completed the whole experiment – participants 002, 006 and 008 and participant 009 stopped at follow-up day 3). Further, it should be noted that the participant from PLAC consumed non-steroidal anti-inflammatory drugs (NSAIDS) during the experiment at 72 hours and throughout the rest of the experiment, despite being instructed to not do so.

Table 3. Participant Characteristics

	BCAA400 (n=3)		**Placebo (n=1)**	
	Pre-experiment	Post-experiment	Pre-experiment	Post-experiment
Height (m)	1.71 ± 0.02		1.65	
Age (years)	21.33 ± 0.58		22.00	
Body mass (kg)	61.15 ± 9.76	61.56 ± 9.71	98.60	99.95
Fat mass (kg)	19.08 ± 11.48	18.92 ± 8.50	37.92	42.09
MVC non-dominant arm (kg)	14.72 ± 1.28	14.37 ± 1.50	17.15	17.10

m = metres, kg = kilograms. Data are reported as mean ± SD.

Table 4. Participant characteristics of those who started the experiment (n=8)

	BCAA400 (n=6)		PLAC (n=6)	
	Pre-experiment	Post-experiment	Pre-experiment	Post-experiment
Height (m)	1.70±0.02		1.63±0.04[1]	
Age (years)	21.00±0.82		21.50±2.65[1]	
Body mass (kg)	60.57±8.05	61.56±9.71	68.66±20.31[1]	99.95±0.00[2]
Fat mass (kg)	17.90±9.66	18.92±8.50	24.72±11.78[1]	46.60±0.00[2]
MVC non-dominant arm (kg)	14.40±1.22	14.37±1.50	14.19±2.43[1]	17.10±0.00[2]

[1] n=6, [2]n=1, m = metres, kg = kilograms. Data are reported as mean ± SD.

4.2 Ear Canal Temperature

Body temperature increased after the ECC exercise (time 0) for both BCAA400 and PLAC, but appeared to be reduced with BCAA. Following the NSAIDS temperature decreased with PLAC (Figure 14). When looking at the individual data, body temperature of all four participants showed a similar trend.

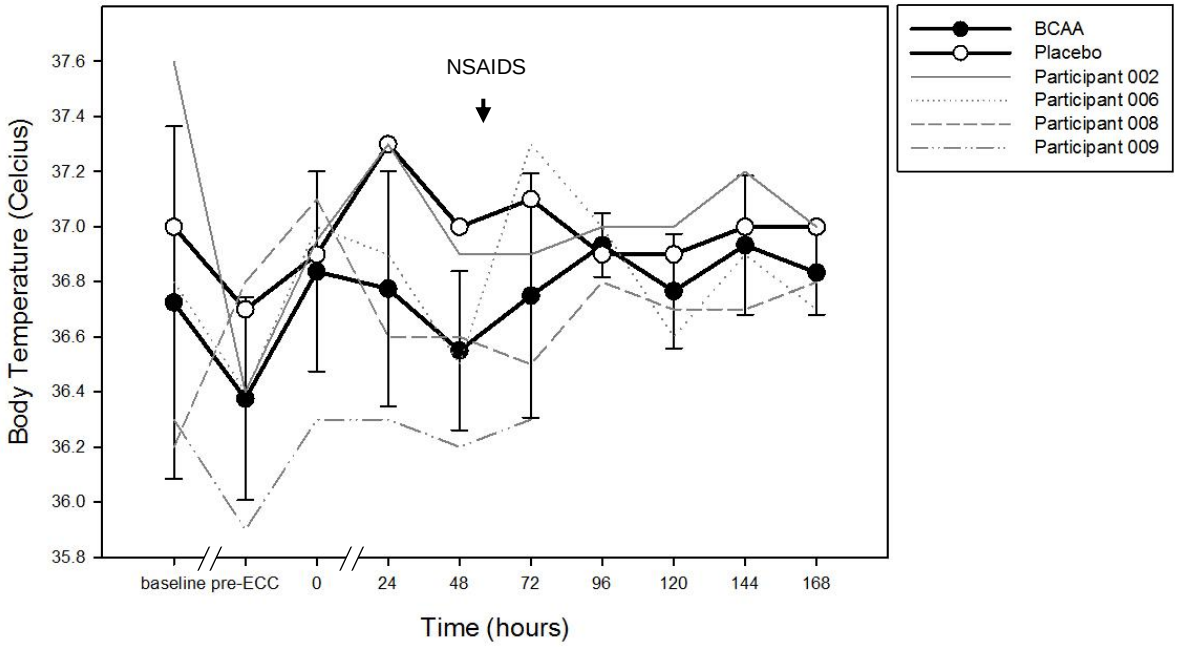

Figure 14. Body temperature (ear canal) throughout the experiment. Baseline measurements were taken 12 days pre-eccentric exercise; pre-ECC was right before eccentric exercise; 0 was immediately after eccentric exercise. Values are means ± SD for BCAA400 (filled circles) and PLAC (open circles). Gray lines represent BCAA individual participant data.

4.3 Limb Girth

Limb girth of BCAA400 appeared to increase slightly right after the muscle damaging exercise (time 0) and remained elevated throughout the experiment (Figure 15). Participants from BCAA400 and PLAC responded similarly despite the consumption of NSAIDS. The PLAC participant had a bigger arm than all of the BCAA400 participants.

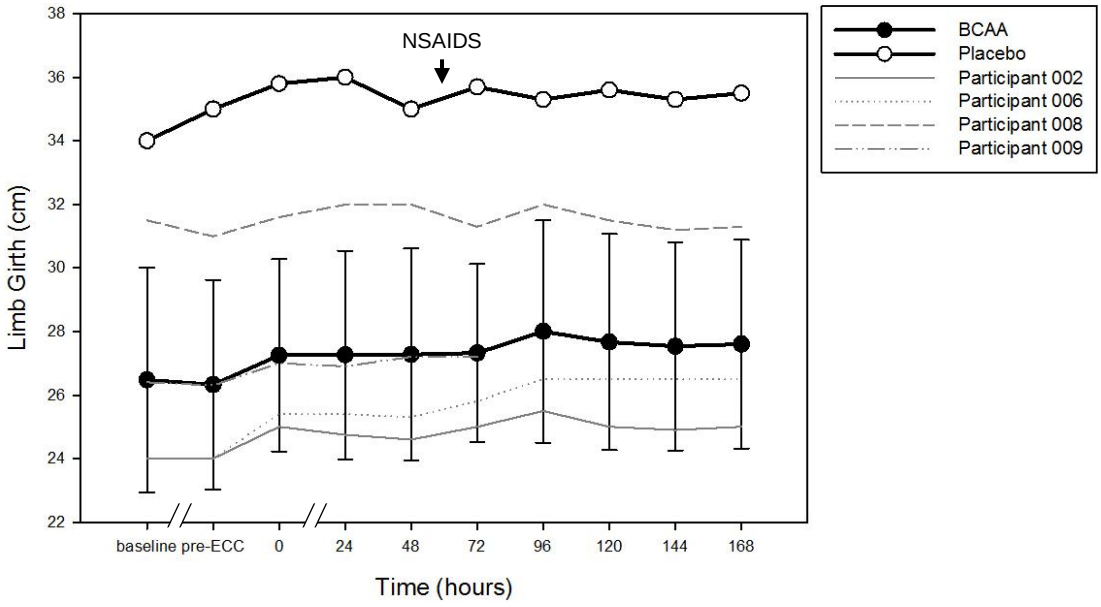

Figure 15. Limb girth throughout the experiment. Baseline measurements were taken 12 days pre-eccentric exercise; pre-ECC was right before eccentric exercise; 0 was immediately after eccentric exercise Values are means ± SD for BCAA400 (filled circles) and PLAC (open circles). Gray lines represent BCAA individual participant data.

4.4 Range of Motion (ROM)

As expected, all participants started with a full range of motion (180º) prior to the damaging exercise, regardless of group. BCAA400 ROM decreased following the exercise, worsened overnight, and slowly recovered almost completely over 168 hours (Figure 16). PLAC followed a similar pattern but seemed to recover fully by 72 hours.

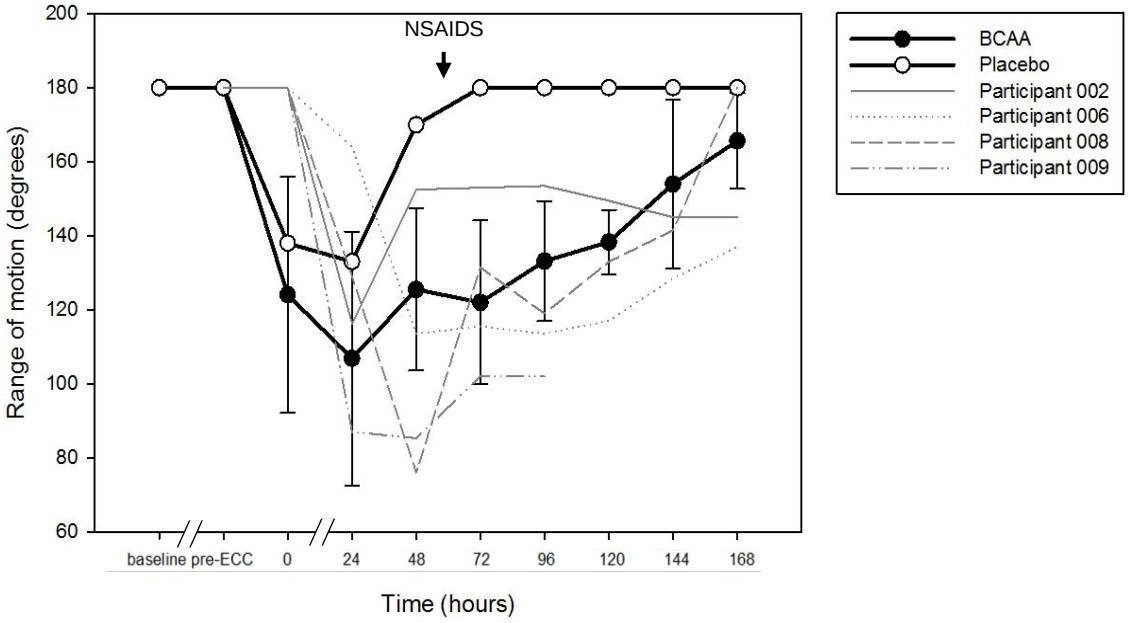

Figure 16. Range of motion throughout the experiment. Baseline measurements were taken 12 days pre-eccentric exercise; pre-ECC was right before eccentric exercise; 0 was immediately after eccentric exercise Values are means ± SD for BCAA400 (filled circles) and PLAC (open circles). Gray lines represent BCAA individual participant data.

4.5 Pain Pressure Threshold (PPT) - relaxed

PPT measures are related inversely to soreness in the muscles, i.e., sore muscles are more sensitive to pain. PPT decreased right after the muscle damaging exercise in both groups and the muscles appeared to be most sensitive after ~48hrs (Figure 17). Thereafter, recovery was nearly linear with BCAA400. Recovery for PLAC was more variable perhaps affected by the NSAIDS intake. Individual data shows a similar trend as the mean of the BCAA group except for participant 008 who did not return to baseline after 168 hours.

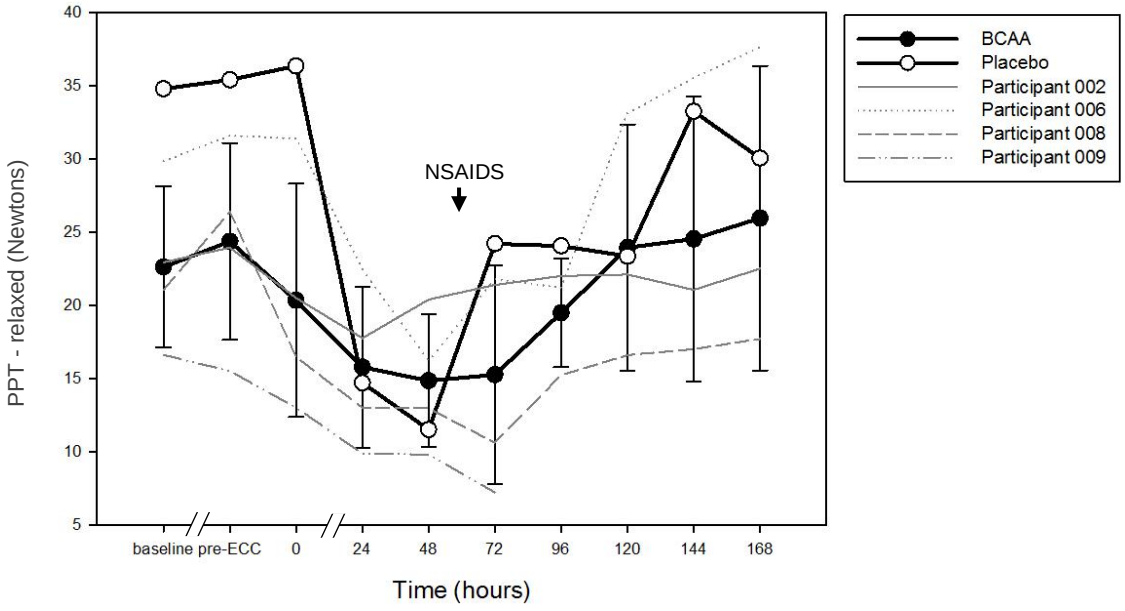

Figure 17. Muscle PPT (relaxed muscle). Baseline measurements were taken 12 days pre-eccentric exercise; pre-ECC was right before eccentric exercise; 0 was immediately after eccentric exercise Values are means ± SD for BCAA400 (filled circles) and PLAC (open circles). Gray lines represent BCAA individual participant data.

4.6 Pain Pressure Threshold (PPT) - contracted

PPT measurements taken when the muscles were contracted indicate a similar trend as the relaxed muscles. Muscle PPT decreased after the damaging exercise bout and continued to decrease until 48 hours post for BCAA400 and 72 hours post for PLAC (Figure 18). BCAA400 returned to baseline by 168 hours. Individual data also shows a similar trend as the mean data.

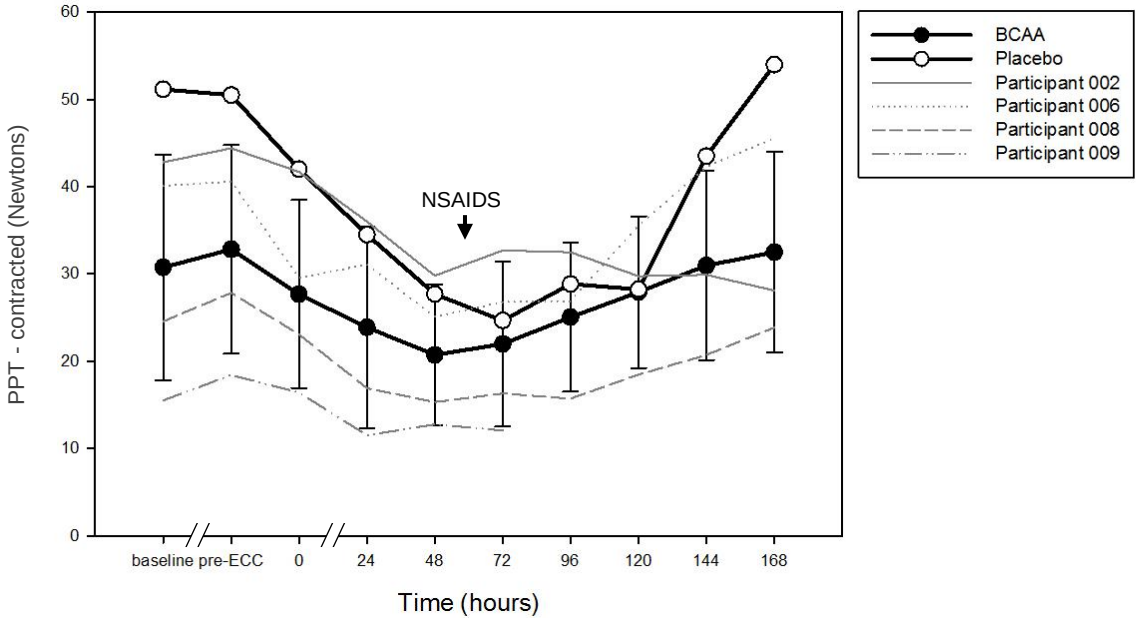

Figure 18. Muscle PPT (contracted muscle). Baseline measurements were taken 12 days pre-eccentric exercise; pre-ECC was right before eccentric exercise; 0 was immediately after eccentric exercise Values are means ± SD for BCAA400 (filled circles) and PLAC (open circles). Gray lines represent BCAA individual participant data.

4.7 Muscle Soreness – Visual Analog Scale (VAS)

The VAS is a 1-10 scale where 1 means no pain and 10 means extremely painful. All participants from both groups self-rated no pain prior to muscle damaging exercise bout. VAS peaked right after ECC, decreased steadily for both BCAA400 and PLAC thereafter (Figure 19). VAS of 2 BCAA participants peaked right after ECC while the other 2 peaked at 48 hours after ECC. If anything, VAS soreness recovered faster with PLAC perhaps due to the NSAIDS.

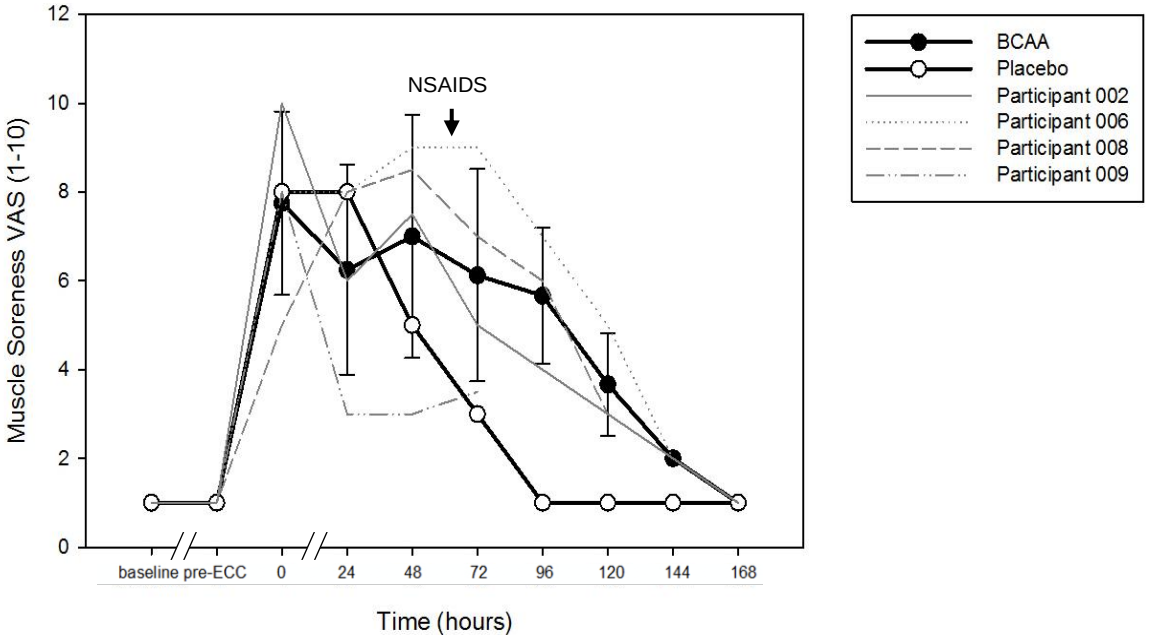

Figure 19. Self-rated muscle soreness throughout the experiment. Baseline measurements were taken 12 days pre-eccentric exercise; pre-ECC was right before eccentric exercise; 0 was immediately after eccentric exercise Values are means ± SD for BCAA400 (filled circles) and PLAC (open circles). Gray lines represent BCAA individual participant data.

4.8 Maximum Voluntary Contraction (MVC)

As expected, MVC decreased after ECC and did not return to baseline even after 168 hours post ECC with BCAA400. MVC returned to baseline for PLAC perhaps because of individual differences or due to the NSAIDS (Figure 20). When looking at the individual BCAA data, a reduction of force can be seen in all participants after ECC. However, the response was quite variable with two participants from BCAA400 returning to baseline after 168 hours while one participant experienced very little recovery even up to 168 hours of recovery.

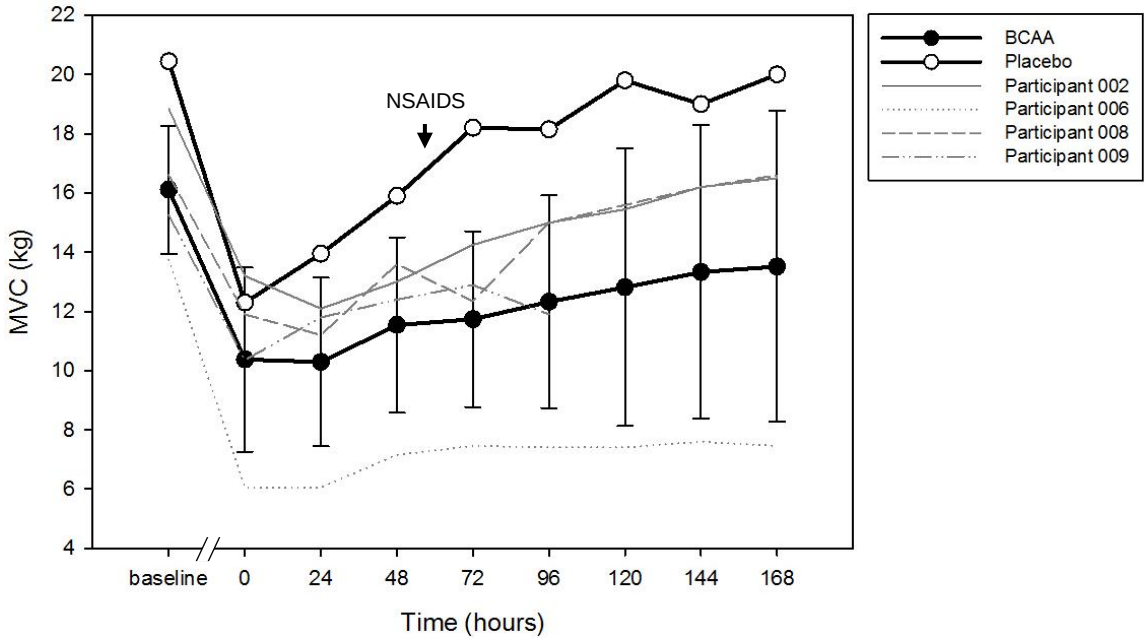

Figure 20. Maximum voluntary contraction (MVC) throughout the experiment. Baseline measurements were taken 12 days pre-eccentric exercise; 0 was immediately after eccentric exercise Values are means ± SD for BCAA400 (filled circles) and PLAC (open circles). Gray lines represent BCAA individual participant data.

4.9 Ultrasound Echogenicity – Biceps

Ultrasound echogenicity (brightness) in the biceps increased following ECC for BCAA400, peaking at 96 hours and then returned to baseline by 144 hours (Figure 21). For PLAC, biceps echogenicity remained constant (near baseline) throughout the experiment.

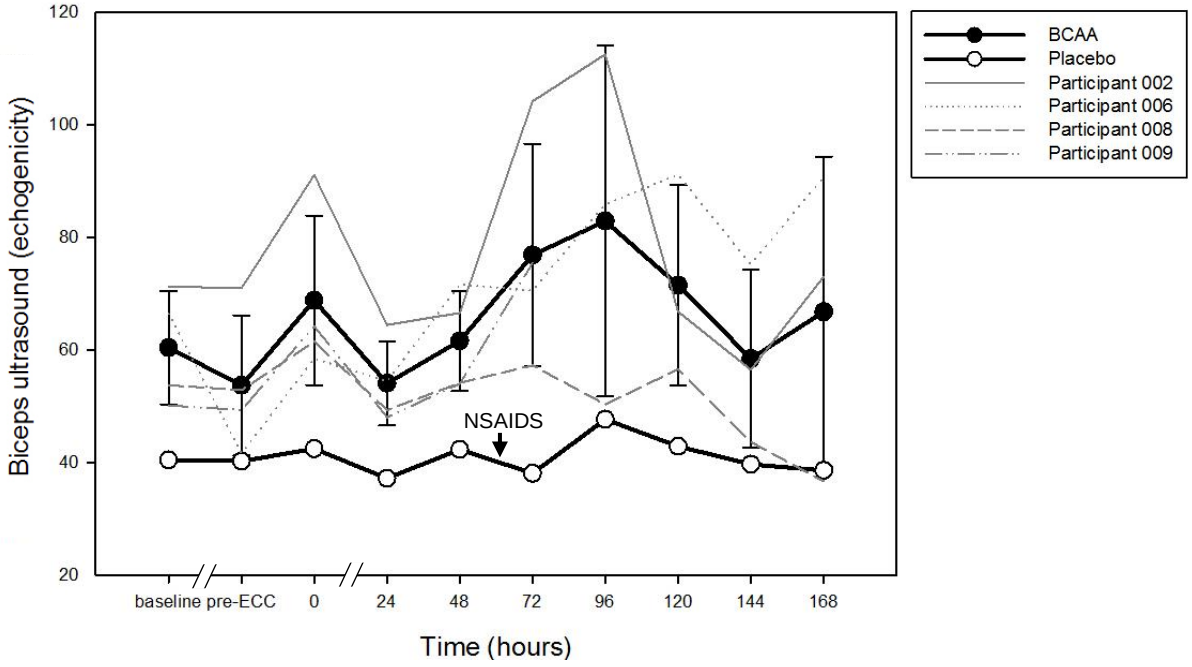

Figure 21. Ultrasound echogenicity of the biceps throughout the experiment. Baseline measurements were taken 12 days pre-eccentric exercise; pre-ECC was right before eccentric exercise; 0 was immediately after eccentric exercise Values are means ± SD for BCAA400 (filled circles) and PLAC (open circles). Gray lines represent BCAA individual participant data.

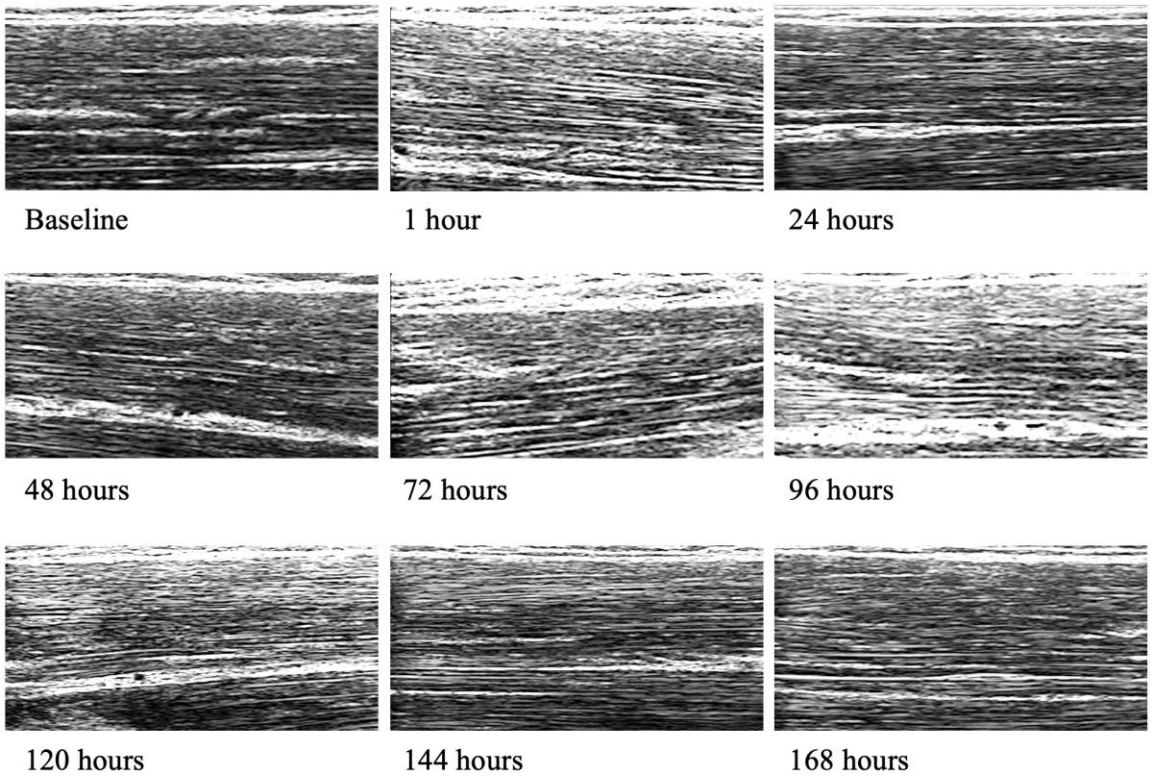

Figure 22. Ultrasound images of the biceps from Participant 002 in BCAA400 throughout the experiment.

4.10 Ultrasound Echogenicity – Brachialis

The echogenicity (brightness) of the brachialis increased gradually post ECC in both groups peaking at 72 and 96 hours for BCAA400 and PLAC, respectively. Both groups returned to baseline at about 120 hours (Figure 23).

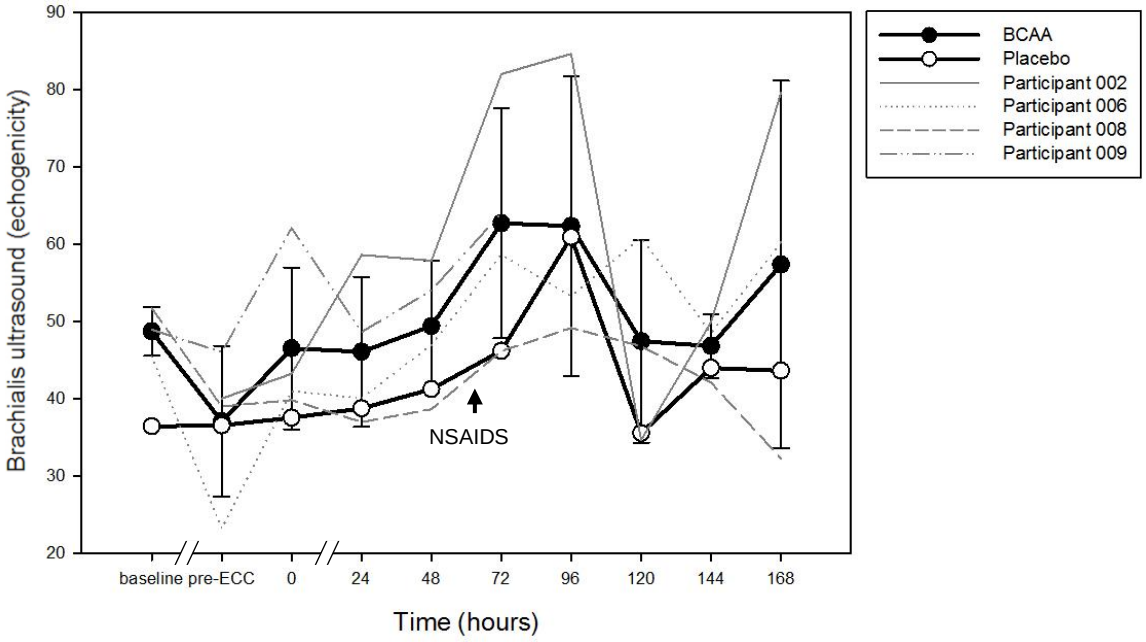

Figure 23. Ultrasound echogenicity of the brachialis throughout the experiment. Baseline measurements were taken 12 days pre-eccentric exercise; pre-ECC was right before eccentric exercise; 0 was immediately after eccentric exercise Values are means ± SD for BCAA400 (filled circles) and PLAC (open circles). Gray lines represent BCAA individual participant data.

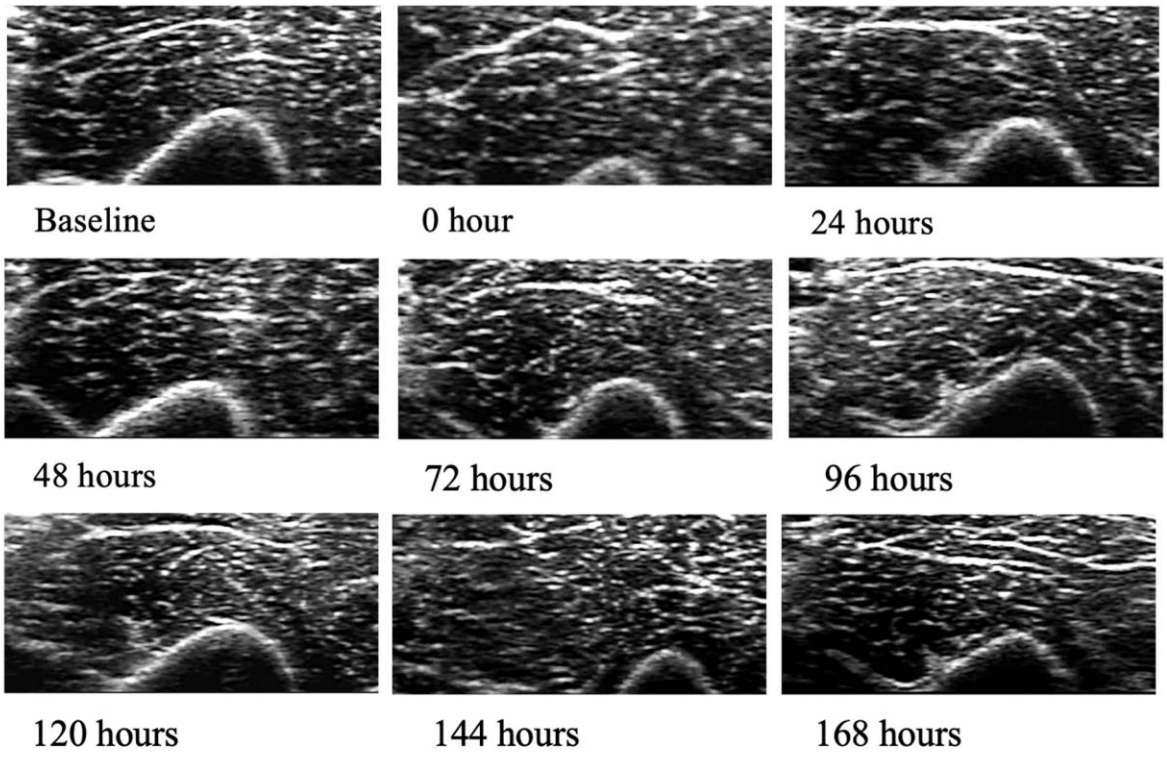

Figure 24. Ultrasound images of the brachialis from Participant 008 in BCAA400 throughout the experiment.

4.11 Rate of Perceived Exertion (RPE)

RPE remained relatively high for both groups throughout recovery from the muscle damaging exercise bout except at 24 hours for PLAC. Individual data indicate that BCAA400 exerted force at or near their maximum throughout the whole follow-up section. In contrast, the PLAC participant rated the 24-hour MVC much lower (Figure 25).

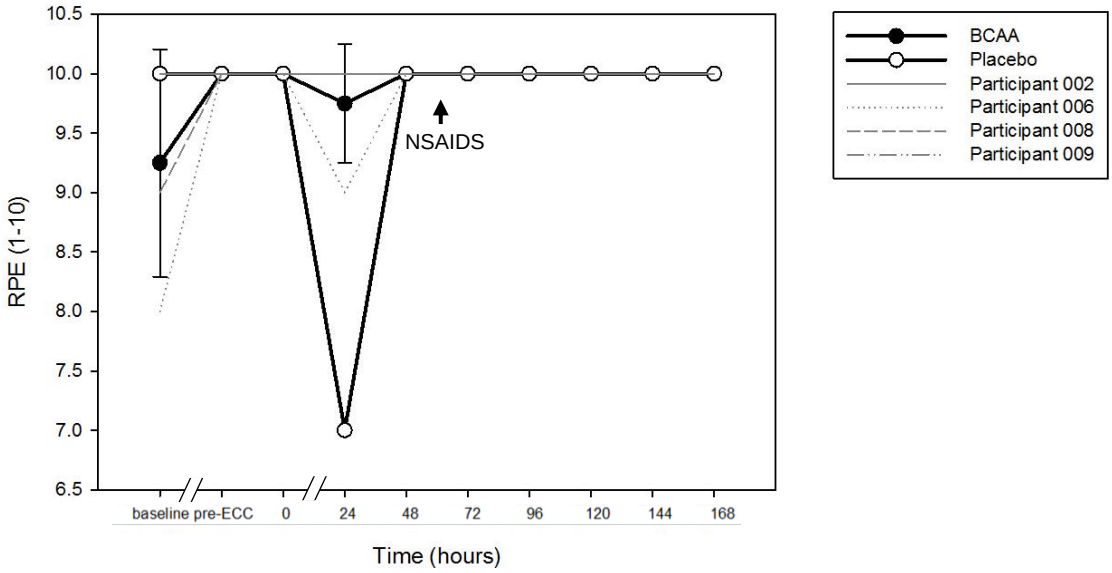

Figure 25. Rate of perceived exertion (RPE) throughout the experiment. Baseline measurements were taken 12 days pre-eccentric exercise; pre-ECC was right before eccentric exercise; 0 was immediately after eccentric exercise Values are means ± SD for BCAA400 (filled circles) and PLAC (open circles). Gray lines represent BCAA individual participant data.

Chapter 5

5 Discussion

The purpose of this study was to investigate the effects of 400mg•kg^{-1}•day^{-1} of BCAA supplementation 12 days prior to a bout of ECC exercise and 7 days following on EIMD and DOMS in young women who had no experience with strength training. Muscle damage was induced by performing 3 sets of 15 reps of ECC exercise (70% of 1RM). The effects of BCAA versus PLAC supplementation were compared over a 7day recovery period to determine if whether BCAA can attenuate the impact of EIMD and DOMS caused by ECC.

Previous studies have shown that ECC causes muscle damage and soreness, resulting in reduced ROM and MVC, with increases in both muscle soreness and ultrasound echogenicity (Hody et al., 2019; Proske & Allen, 2005; Whitehead et al., 1998). Previously, both isokinetic muscle force measurement (Agru et al., 1987; Noreau & Vachon, 1998) and MVC measurement (Peake et al., 2017) have been utilized to examine the reduction of muscle force caused by EIMD. Consistent with the literature, the results from our experiment indicated a decline in MVC right after ECC (time point 0 hours on graphs) and throughout 7 days of follow-up. Further, VanDusseldorp and colleagues (2018) reported that ROM dropped by 31% in BCAA400 and 23% in PLAC following a muscle damaging protocol. Our range of motion results were similar. Other EIMD parameters including objective and subjective muscle soreness measurements using the PPT and VAS respectively, and the echogenicity of ultrasound images also showed evidence of muscle damage and soreness. Together these data suggest that elbow flexor muscles were damaged due the ECC performed in our study. As the participant who completed the PLAC trial consumed NSAIDS between 48 to 72 hours after ECC, data prior to the timepoint 72 hours should reflect the traits of muscle damage and soreness but data at 72 hours and beyond are likely affected. Therefore, data prior to that timepoint will be discussed in the following section; however, of course, as there is only one PLAC participant and considerable inter-interindividual variability, comparisons are limited.

5.1 Effects of BCAA on MVC

As mentioned, the muscle damaging ECC protocol was successful as all participants showed signs of EIMD and DOMS after ECC. MVC dropped by 36% and 40% immediately after ECC when compared to baseline in the BCAA400 and PLAC, respectively. The PLAC participant followed a similar MVC recovery pattern but seemed to recover more quickly even before the NSAIDs use. This might be due to individual differences. Previously Howatson and colleagues (2012) supplemented male competitive national league rugby and football athletes with placebo or 10g of BCAA in the morning and 10g in the evening for 12 days. They found an 18% decline in MVC 24 hours post damaging protocol in the BCAA group and a 27% decline in the placebo group. Others have reported similar declines in MVC by 31% and 29% for their BCAA and placebo groups, respectively (Fouré et al., 2016). Similar to the literature, our study shows a decline in MVC immediately after ECC and MVC did not return to baseline at 168 hours post ECC. Future studies should examine the decline in MVC with different doses of BCAA supplementation in both males and females as females tend to have a greater reduction and faster recovery in MVC when compared to males (Sayers & Clarkson, 2001). Moreover, age seems to be a factor that affects submaximal isometric force after ECC with different muscle groups (Enoka et al, 2003; Lavender & Nosaka, 2007). Therefore, the effects of BCAA supplementation on MVC recovery post-ECC should be studied in both genders, in different age groups and in different muscle groups.

5.2 Effects of BCAA on Objective (PPD) and Subjective (VAS) Muscle Soreness Measurements

The mammalian target of rapamycin (mTOR) signalled by leucine (Li et al., 2011) stimulates muscle protein synthesis (Drummond et al., 2009) and leucine can also reduce pro-inflammatory cytokines production (Allan, 2008). Since secondary damage after ECC is triggered by inflammation (Howatson & van Someren, 2008), a reduction in inflammatory cytokines may reduce secondary damage. Hence, minimizing the effect of

EIMD and DOMS. Consequently, leucine could reduce inflammation and/or speed repair of any damage associated with ECC. Our objective muscle soreness - PPT sensitivity increased by 10% and 10% for BCAA400 and 18% and 10% for PLAC when the measurements were taken when participants contracted or relaxed their muscles immediately post ECC, respectively. Our subjective muscle soreness measured by VAS also increased post ECC in both groups, which also agrees with previous studies (Fouré et al., 2016; Greer et al., 2007; Shimomura et al., 2010; VanDusseldorp et al., 2018).

The PPT (both relaxed and contracted) measurements suggest that PLAC experienced greater discomfort and muscle soreness than BCAA400 despite the consumption of NSAIDS, although the latter appeared to influence the response. Further, the PLAC participant told us that she has a high pain tolerance which is consistent with the observed high pain tolerance at baseline. Participants were asked to relax and contract their muscles during PPT measurements and data from both relaxed and contracted muscles shows a similar trend. Future studies could just perform measurements when the muscles are relaxed or flexed instead of doing both. However, immediately after ECC, some participants made remarks about not being able to contract their biceps due to soreness and fatigue, which can lead to inconsistencies in data collection. Hence, the recommendation for future studies is to select preforming PPT measurements when the muscles are relaxed to reduce errors.

Moreover, VAS peaked immediately after ECC for both BCAA400 and PLAC, indicating an increase in perceived muscle soreness. BCAA400 appeared to return to baseline at 168 hours post ECC while PLAC returned to baseline at 96 hours, which could be caused by NSAIDS consumption. In previous studies, VAS did not return to baseline at 4 days post damaging exercise (Fouré et al., 2016; Howatson et al., 2012; Ra et al., 2013), nor 5 days post damaging exercise (Shimomura et al., 2010). In our study, a lowered PPT value suggests increased muscle sensitivity with EIMD. The observed PPT had a positive linear relationship with ultrasound echogenicity, VAS and an inverse relationship with MVC and ROM. For example, as PPT decreased after ECC due to increased soreness and EIMD, ultrasound echogenicity increased due to edema and inflammation. At the same time, VAS increased indicating greater subjective soreness.

VAS has been widely used as a tool to quantify pain from DOMS due to the ease of use (Slater et al., 2010). On the other hand, PPT has been used previously to quantify objective pain as well as to compare pain threshold in individuals with chronic neck pain (Walton et al., 2011), and individuals with hypertension-type headaches and migraines (Andersen et al., 2015). Although these two measures can both provide data on pain, the relationship between PPT and VAS has yet to be determined. For instance, a previous study has shown the that the correlation of PPT and VAS is not significant (Lau, Muthalib & Nosaka, 2012) while another study suggests the opposite (Alfonsin et al., 2019). So, although both VAS and PPT have considerable promise, more research is required to determine the relationship between PPT and VAS, as well as to determine the best measure of soreness, whether it be PPT, VAS or a combination of both measurements.

5.3 Effect of BCAA on Ultrasound Echogenicity

Biceps ultrasound echogenicity increased by 14% and dropped by 4% in the brachialis echogenicity for BCAA400, while echogenicity increased in both the biceps and brachialis by 5% and 3% in PLAC immediately post ECC. This was expected as surges in echogenicity can be caused by the increased local muscle temperature and greater blood flow to exercising muscles (Beitler et al., 1983). To our knowledge, this is the first study to perform ultrasound echogenicity measurements throughout the follow-up after an ECC bout with BCAA supplementation. Echogenicity results from the biceps were greater throughout the whole experiment for BCAA400 than PLAC. However, as observed in the ultrasound images in this study, PLAC has a thicker layer of fat tissue when compared to BCAA400 participants. As a result, this apparent greater body fat composition in PLAC may have been a factor as echogenicity can be reduced by body fat percentage (Mazhar et al., 2008) due to the hypoechoic properties of fat tissue (Spencer at al., 1995). BCAA400 had higher overall echogenicity in the biceps while PLAC has higher overall echogenicity in the brachialis. By looking solely at the results of the brachialis, PLAC performed worse immediately after, 24 hours post ECC and throughout the experiment despite the

consumption of NSAIDS which would be expected to reduce perceived muscle soreness and pain. Future studies could utilize skin fold measurements at the biceps to match participants and to reduce variations between participants.

Additionally, the ultrasound technician needs to be experienced in order to capture useful images. They should be able to tell from the image captured where the necessary anatomical structures for analysis are and determine if that image is useful. Furthermore, the image quality was better in the biceps images as it is easier to capture images there when compared to the brachialis. This is because the biceps is more superficial while the brachialis is deep. Therefore, the technician should know the anatomy well so that proper images of the brachialis can be taken. In addition, an objective repeat image analysis to assess reproducibility (several ultrasound images should be analyzed blindly, multiple times) is always wise.

Regardless, our results, show that the echogenicity of biceps and brachialis provided similar trends. Therefore, investigators in future elbow extension muscle damaging studies who would like to take ultrasound images but do not have the experience in ultrasound imaging are recommended to only capture images from the biceps. Moreover, experienced ultrasound technicians in future studies could also measure muscle thickness and pennation angle in response to EIMD.

5.4 Limitations

The major limitation of this study is the small sample size due to the inability to collect data because of COVID-19. This is especially so as there was only one PLAC participant, who was bigger physically that the BCAA participants. Further, her use of NSAIDS make comparisons difficult. Also, there was some variability in menstrual cycle length from that estimated from previous cycles (one participant reported her menstrual cycle started one day early and another started one day later) even though all participants reported that they had consistent menstrual cycles routinely. This might have been caused by the stress of the experiment or final exams which were upcoming (Jacobson, et al.,

2012) or other underlying health conditions. Regardless, this should not be a major concern as all participants performed ECC during the follicular phase and hormonal fluctuations would not be expected to vary significantly one day prior to or one day post the estimated start of the menstrual cycle.

The current study utilized elbow extension with dumbbells to damage the muscles, and a metronome to standardize the velocity of ECC contractions, which has been done in other studies (Nosaka et al. 2006; VanDusseldorp et al., 2018). Although the above method is more accessible and less expensive when compared to other types of ECC exercise, we cannot rule out the possibilities of human errors during data collection. Future studies could also utilize an incline preacher bench setup to standardize the ECC protocol better. Participants were asked to resist the weight of the dumbbell and tried to reach full elbow extension in 4 seconds, and they were allowed to practice before the muscle damaging protocol without using any weight. They were able to familiarize with the timing of elbow extension, but when it comes to the actual damaging exercise with dumbbells, participants found it challenging to resist the dumbbells and to extend the elbow at a certain velocity. The velocity of elbow extension could vary due to individual differences. If so, this could be important as previous studies have shown that the velocity of ECC contractions can alter the degree of muscle damage (Katz, 1939; Proske & Morgan, 2001). However, this was not a major concern for our experiment as all participants were able to complete a full elbow extension in close to four seconds. Future studies should consider other ECC protocols to better standardize the velocity of ECC contractions.

In addition, muscle damage can lead to an increase in core temperature due to inflammation (Montain et al., 2000) and physical stress (Fraga et al., 2020). In the current study, ear canal temperature may not have reflected the increased muscle temperature after eccentric exercise. However, participants and investigators were able to sense an increase in local muscle temperature after ECC and throughout the follow-up period. The use of muscle or even skin temperature might have been better to assess any changes in temperature. Therefore, future studies should take into account these limitations to reduce variation among participants.

5.5 Summary and Conclusion

Sixteen untrained female participants with no experience in strength training were recruited for this study but only four participants completed the whole exercise protocol due to a worldwide pandemic COVID-19. Participants consumed either 400mg•kg^{-1}•day^{-1} of BCAA or placebo (fibre) for 12 days prior to a bout of eccentric exercise and throughout seven days of follow-up. Data on EIMD parameters were collected before supplementation, before and immediately after eccentric exercise, and the over seven days of follow-up.

The current data suggest that muscle damage occurred with the ECC exercise studied but identifying any effects of BCAA is not possible due to the small number of individuals studied. This is especially true because there was only one PLAC participant, who was larger than the BCAA individuals, and who took NSAIDS during the recovery period. Specifically, arm girth, body temperature, muscle soreness, and ultrasound echogenicity increased, while MVC and ROM decreased following the ECC exercise in both groups. It is interesting to note that both the objective and subjective muscle soreness ratings for the PLAC participant were greater than BCAA, despite NSAIDS consumption, which is consistent with a BCAA effect in reducing soreness in muscles but less effective in force recovery. However, no obvious group differences were observed consistently. Therefore, whether BCAA supplementation can attenuate the decline of MVC immediately following ECC and/or reduce DOMS must wait further study.

5.6 Future Directions

This study cannot determine whether 400mg•$^{-1}$kg•day^{-1} of BCAA supplementation influences muscle damage or soreness after a bout of eccentric contractions at 75% of their 1RM. Future studies could use a within-subject design and more participants, although this would require a washout period (several weeks) between treatments due to the repeated bout effect (Rawson et al., 2004). Moreover, changes in energy intake, protein consumption, or menstrual cycle timing (circulating progesterone and estrogen)

could affect recovery after a bout of eccentric exercise via effects on whole body protein turnover (Toth et al., 2006), leucine oxidation (Lariviere et al., 1994) and/or muscle strength (Sarwar et al., 1996). As shown by Hamadeh and colleagues (2005), eight days of estrogen supplementation in recreationally active men reduced both CHO and leucine oxidation and increased lipid oxidation throughout the course of an endurance exercise bout. Thus, diet and standardization of experimental protocols in accordance with menstrual cycles need to be controlled in future experiments. Further, male participants also need to be studied and the relationship between BCAA and EIMD should be investigated on other exercise movements targeting different muscle groups including real-life exercise situations. Finally, the effect of BCAA on EIMD can be affected by sex, exercise experience, types of exercise performed and underlying health conditions, etc. Therefore, future research might be improved if it was focused on individual differences.

To our knowledge, this is the first study to utilize a PPD to assess the muscle PPT after a bout of eccentric exercise on healthy young female adults with BCAA supplementation. Walton and colleagues (2011) have shown that individuals without experience in PPT measurements were able learn the technique easily and to collect accurate data. Combined with our observations, this suggests that a PPD is a valuable measuring tool to quantify muscle soreness/pain after muscle damaging exercise. Therefore in future studies investigators should consider using it to quantify muscle pain and soreness objectively.

Additionally, some have reported that BCAA supplementation can reduce depression (Baranyi et al., 2016) and improve sleep quality (Gehrman et al., 2018). It is known that exercise can reduce depression (Craft & Perna, 2004) and improves quality of sleep (Dolezal et al., 2017) so combining BCAA supplementation with exercise should be studied to determine if this combination is better than either alone for this patient group.

Finally, to examine the relationship between inflammation and muscle adaptation, NSAIDS might be a useful tool. Non-aspirin NSAIDS reduce inflammation by inhibiting the synthesis and release of prostaglandins (PG) which are responsible for the initiation of inflammatory response (Weissmann et al., 1987). Interestingly, BCAA have many

similar effects and this may be responsible for the observed anti-inflammatory response (Bassit et al., 2002; Kato et al., 2016). NSAIDS containing cyclooxygenase enzymes were first used by Vane (1971) as anti-inflammatory agents. Currently, they are the most common drug used by Canadian athletes (Lippi, et al., 2006). A common type of NSAIDS is ibuprofen, which is commercially available and has been widely used to reduce inflammation and pain. Importantly, a study done by Krentz and colleagues (2008) showed that moderate ibuprofen consumption ($400mg \bullet day^{-1}$) did not limit muscle strength or hypertrophy gains during six weeks of strength training in experienced weightlifters. However, Lilja and colleagues (2017) suggest that a high dose of ibuprofen ($1200mg \bullet day^{-1}$) attenuated muscle strength and hypertrophy after eight weeks of knee-extensor strength training in recreational athletes. Several differences between the studies include the dosage and the training status of participants could explain the varied results. Animal study results indicate that reducing the influx of macrophages into the damaged muscles will impair the regeneration and repair of muscles (Summan et al., 2006). Therefore, it appears that proper regeneration of stressed or injured muscles require a macrophage response (Butterfield, Best & Merrick, 2006). In addition, strength training can activate a response to blunt pro-inflammatory responses (Gordon et al., 2012). Hence, unlike untrained individuals, strength training does not initiate an inflammatory response in trained individuals. In fact, it can provide anti-inflammatory benefits (Calle & Fernandez, 2010).

In summary, if trained individuals do not require an inflammatory response to initiate muscle hypertrophy and strength gains while untrained individuals do, NSAIDS consumption will likely affect strength gains and hypertrophy in trained vs untrained individuals differently, i.e., trained individual could consume NSAIDS to alleviate pain, but untrained individuals should not. Perhaps trained individuals have a different underlying mechanism explaining muscle hypertrophy and strength gains when compared to untrained individuals. Consequently, it would be interesting to investigate whether different doses of NSAIDS consumed by untrained and trained individuals produce different results. More research is needed to assess the impact on muscle strength and hypertrophy when inflammation is attenuated in both trained and untrained individuals.

Regardless, NSAIDS could have an impact on muscle damage and inflammation and need to be controlled in future experiments.

Chapter 6

6. Proposal

The Optimum Dose of Branched-Chain Amino Acid Supplementation on Muscle Damage Makers in Sedentary Adults

6.1 Introduction and Significance

Branched-chain amino acids (BCAA) are a common nutrient supplement that is being consumed presently by athletes at all levels (Fouré & Bendahan, 2017). This is occurring largely because it has been documented that dietary leucine can reduce muscle protein degradation as well as stimulate muscle protein synthesis (Leenders & van Loon, 2011). However, previous BCAA supplementation studies have not been able to show consistent effects. Most importantly, the amount and duration of BCAA supplementation is variable and the degree of muscle damage among studies has not been controlled. Regardless, based on a meta-analysis (Fouré & Bendahan, 2017), it appears that the minimum effective BCAA dose is ~200mg BCAA•kg^{-1}•day^{-1} for at least 10days before the damaging exercise bout. This proposed experiment is designed with these considerations in mind. Specifically, we plan to standardize the exercise bout and compare the effect of different doses of BCAA on muscle damage markers in healthy recreationally active individuals. This work should provide valuable information regarding the dosage of BCAA needed to reduce muscle soreness associated with occupational or recreational muscle damage.

6.2 Objectives and Hypothesis

Three groups supplementing with BCAA (0-500mg•kg^{-1}•day^{-1}) will be studied following standardized, muscle damaging exercise. We hypothesize that 12d BCAA

supplementation beyond 200mg•kg^{-1}•day^{-1} will reduce the magnitude of delayed onset of muscle soreness (DOMS). Further, we expect to observe a dose response effect for muscle damage.

Specifically, blood myoglobin, 3-methyl-histidine and insulin concentration, muscle temperature, limb girth and VAS will be lower in all BCAA groups in a dose dependent manner vs PLAC. MVC will be greater in the BCAA groups vs PLAC while ultrasound images for PLAC will be more hyperechoic than the BCAA groups. RPE will be unchanged.

To investigate, we will explore the effect of different doses of BCAA consumption on exercise-induced muscle damage (EIMD). Participants will consume various doses of BCAA or PLAC 12days prior to a bout of eccentric exercise and throughout a 7-day follow-up period. To test the effect of BCAA supplementation on exercise-induced muscle soreness the main outcome measure will be maximum voluntary contraction (MVC) because it is known to decrease in proportion to soreness. Participants will perform downhill running for 45 minutes and measurements will be made 12 days before BCAA or placebo supplementation (baseline), as well as at 1, 3, 24, 48, 72, 96, 120, 144 and 168 hours after muscle damage. Greater soreness will result in reduced MVC.

6.3 Background

According to Statistics Canada (2017), 46% of Canadians have consumed at least one nutritional supplement. Among the supplements, protein is popular in athletes to boost recovery or to gain muscle mass. Unlike protein consumption, there is no daily recommended intake nor upper limit for BCAA to increase muscle mass or to reduce EIMD. Previous studies have shown 12 days of BCAA supplementation can reduce EIMD markers after cycling for 120 min at 70% VO_2 max (Coombes & McNaughton, 2000). In contrast, 7-day supplementation prior to a marathon run was not able to prevent muscle damage (Areces et al., 2014). The current literature does not provide an optimal BCAA dose due to differences in the amount of BCAA consumed, duration of

supplementation, exercise training experience of individuals as well as the type of exercise being performed to damage muscles. However, Fouré and Bendahan (2017) suggested in a meta-analysis that individuals should consume more than 200mg•kg^{-1}•day^{-1} of BCAA for at least 10 days in order to mitigate muscle damage. We aim to confirm this observation as well as search for the optimal dosage for muscle recovery from a standardized bout of eccentric (ECC) exercise.

6.4 Research Methods and Preliminary Data

6.4.1 Participants

78 healthy sedentary men and 78 sedentary women between the ages of 18 to 29 will be assigned to three treatment groups of 26 to participate in a 18-day BCAA supplementation program: ($n_1 = 26$; $n_0 = \frac{2\times(SD)^2\times(0.84+1.96)^2}{(difference\ of\ the\ means)^2}$; $n_0 = \frac{2\times(273)^2\times(0.84+1.96)^2}{(976-734)^2}$; $n_0 = 20$; assuming a 30% dropout, $n_1 = 20 + (20 \times 30\%)$; $n_1 = 26$). (Waldon et al., 2017). Participants will be supplemented with either 0, 250 or 500 mg BCAA•kg^{-1}•day^{-1}or (placebo, fibre supplement) for 12days before inducing muscle damage on the knee extensors. All groups will be matched based on body mass of the participants (males 70±5kg; females 60±5kg) and biological sex. Participants will be excluded if they are diabetic or have been using protein supplements, drugs, or have a history of cardiovascular disease, joint and/or muscle injuries. Female participants will be excluded if their menstrual cycle is irregular and/or if they take oral contraceptives. Participants will avoid alcohol, anti-inflammatory drugs, use other therapeutic interventions (such as massage and hydrotherapy) and the use of compression garments during the intervention period. All volunteers will be asked to complete a medical screening questionnaire and a written informed consent will be obtained before any participation.

6.4.2 Experimental overview

Three treatment groups will be assigned to be supplemented with either 0 (placebo, fibre supplement; PL), 250 (BCAA250) or 500 mg BCAA•kg^{-1}•day^{-1}(BCAA500). 78 participants (39 men and 39 women) will be ranked within each sex block separately from 1-39 based on body mass. Then, to keep the male and female blocks homogeneous for body mass, within each block, number 1 will be assigned to Placebo, number 2 to 250 mg, number 3 to 500 mg, number 4 to 500mg, number 5 to 250 mg, and number 6 will be assigned to Placebo. Thereafter, this procedure will be repeated until all participants are assigned. It is not anticipated that recruitment will be problematic but, if so, the study can begin before all 78 participants are enrolled because the body mass restrictions will keep the three treatment groups similar even when done sequentially. Supplements will be provided in a double-blind fashion to be ingested one hour after each of the three main daily meals for 12days. Participants will be asked to refrain from eating any meat products three days prior to the experimental day as it will affect 3-methyl-histidine in blood samples. Anthropometric measurements (body mass, lean mass, height), local muscle temperature of knee extensors (both legs), ultrasound images (US), strength assessment (maximal voluntary contraction, MVC), rate of perceived exertion (RPE), range of motion (ROM), thigh girth, muscle pain measurement and blood samples (duplicates; myoglobin; 3-methyl-histidine; insulin) will be collected before, during and after the supplementation period at different time points. Participants will perform three repetitions of isometric knee extension at their maximal force (1RM) with both their dominant and non-dominant leg on an isokinetic dynamometer (Biodex®) one day before supplementation starts. Participants will arrive at the lab fasted overnight using public transportation or car on all test days. Participants must refrain from aerobic exercise training as delayed onset of muscle soreness (DOMS) can be induced by any exercise with an eccentric component (common with running down hills). Participants can still engage in low intensity exercise such as walking, but moderate and high intensity training such as bicycling, jogging and running must be eliminated. For women the day of exercise-inducing muscle damage will be on the 7^{th} day of their menstrual cycle (early follicular phase).

6.4.3 Measurements

Body mass and lean mass will be measured with Bodpod® and height will be measured with a free-standing stadiometer. Local muscle temperature will be measured over the muscle belly of the quadriceps. Thigh girth (10 and 20 cm proximal to the superior pole of the patella) will be measured with an anthropometric tape when participants lay down in supine position with their muscles relaxed. Ultrasound measurements will be used to scan the knee extensors and can be seen as being hyperechoic when there is muscle damage, where the image of the damaged area will be brighter when compared to the normal surrounding area. Ultrasound images are taken in addition to limb girth to assess muscle damage as the former is a more direct measurement and provides a better view of the damaged muscle. ROM of the knee will be measured by asking the participant sit on a chair with the knee angle standardized at 90 degrees, followed by extension and flexion until they experience pain. A goniometer will then be used to measure the angles. Muscle pain measurements will be measured using a pain pressure dynamometer (PPD). MVC will be measured using a Biodex machine and blood samples will be taken on the non-dominant arm. Subjective muscle soreness will be determined using a visual analogue scale (VAS; 1 being no pain and 10 being extremely painful) as participants fully extend their knee slowly while seated.

6.4.4 Exclusion Criteria

Exclusion Criteria:

- Individuals who cannot complete three days of not eating meat
- Diabetic individuals
- Those recent strength training experience (within the past year)
- Those taking protein supplements (previous 6 months)
- Those consuming a high daily protein intake (> 2g/kg body mass)
- Those taking omega-three or vitamin E supplements in the past year
- Those taking anabolic steroids or other medications that could affect muscle metabolism/soreness

- Those with cardiovascular disease
- Those with a history of significant joint & muscle Injuries
- Females with irregular menstrual cycle and/or take oral contraceptives

Inclusion Criteria:

- Healthy males and females at the age of 18-29y
- Do not have recent (previous year) strength training experience
- Able to read, understand, and communicate in English so they can consent for themselves
- Sedentary

6.4.5 Prior to inducing muscle damage – diet and exercise

Participants must refrain from any exercise at least five days before the supplementation day until the end of the experimental protocol and must refrain from eating any meat products three days prior to the experimental day. A detailed dietary intake will be recorded for three days (two weekdays and one weekend day) with the use of a free phone application (MyFitnessPal®) prior to the supplement intervention period. Participants will have to provide their sex, date of birth, country of residence, height and mass. This information is required so that the application can predict the daily total energy intake. A change in body mass will be problematic for our study as participants could be out of the range of the inclusion criteria (males 70±5kg; females 60±5kg) when there are changes to their body mass. Therefore, participants will be asked to choose to click "maintain body weight". Participants input the food they eat by taking a picture of the food or the food label so that the app can calculate the number of calories, macronutrients and micronutrients in the food. Participants will email their data to the researchers.

6.4.6 Supplementation

Supplements will be provided to participants in a double-blind manner, they will ingest BCAA supplements with a liquid drink mix with zero calories designed to mask the taste of the BCAA. Participants can choose from several flavours. The amount of BCAA supplementation ingested will be based on their body mass ($mg \bullet kg^{-1} \bullet d^{-1}$). Participants will ingest their supplements 1hour after each main meal (breakfast, lunch, dinner). Participants will not be able to distinguish BCAA and fibre supplement by taste, color or scent. Supplementation for females will start 12 days prior to their estimated menstrual cycle.

6.4.7 Measurements prior to ECC

Participants will rest seated for 15 minutes before taking any measurements. Body mass, lean mass, local muscle temperature over the knee extensors will be measured before breakfast, respectively. A standardized breakfast (jelly, containing carbohydrate only; amount will be standardized based on their body mass ($7kcal \bullet kg^{-1}$, approximately 500kcal for a 70kg individual, equal to 25% of the daily recommended intake) and will be provided to all participants on the day of muscle damage. After breakfast, thigh girth, ROM, ultrasound measurements, muscle pain measurements using PPT and VAS will be measured.

6.4.8 Muscle damage induction

Participants will perform a standardized warm-up procedure by walking at 4km/hr on the treadmill at a 15º angle downhill for five minute before the muscle damaging exercise, there will be a three minute rest period between warm-up and the actual exercise bout. For the exercise bout, all five groups will be running (10 km/hour) at a 15º angle downhill for 45 min. MVC will be performed using a Biodex machine. Their rate of

perceived exertion will be examined after each set. Investigators will provide strong verbal encouragements throughout the damaging protocol.

6.4.9 Measurements after ECC

Participants will be asked to determine RPE immediately after the muscle damaging protocol. Muscle soreness, muscle pain, local muscle temperature, MVC, thigh girth, ultrasound images and blood samples will all be taken at 30, 60, 120 minutes, 24, 48, 72, 96, 120, 144 and 168 hours after muscle damage.

6.4.10 Statical Analysis

All data will be expressed as means ± SD. A two-way ANOVA (treatment by time) with repeated measures will be used to compare MVC, myoglobin, muscle soreness, resting metabolic rate and body temperature. Dietary information will be assessed using a one way ANOVA. P will be set at $P < 0.05$. A Tukey honestly significant difference post hoc test will be used to determine the significant differences between means as well as the Bonferroni correction for multiple comparisons.

6.5 Future directions

Future studies should establish an optimum dose of BCAA supplementation for strength trained individuals for older adults, and for individuals who are suffering from diseases that reduce muscle mass.

6.6 Summary and Impact

BCAA supplementation can reduce DOMS caused by EIMD as it can reduce inflammation and swelling of the muscles. This will be the first study to investigate the impact of different doses on DOMS and EIMD, as well as to establish an optimum dose of BCAA consumption to reduce DOMS. Although the upper limit of BCAA has not been established in the literature, an optimum dose will reduce the cost in supplement consumption. Further and importantly, reducing the soreness might encourage more Canadians to exercise, which could help reduce healthcare costs as well as improve the overall health of Canadians.

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Appendices

Appendix A: PAR-Q+

2020 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1) Has your doctor ever said that you have a heart condition ☐ **OR** high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it **does not limit your current ability** to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow Global Physical Activity Guidelines for your age (https://apps.who.int/iris/handle/10665/44399).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION

If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for its records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

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FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?

If the above condition(s) is/are present, answer questions 1a-1c — If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐

2. Do you currently have Cancer of any kind?

If the above condition(s) is/are present, answer questions 2a-2b — If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm

If the above condition(s) is/are present, answer questions 3a-3d — If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐

4. Do you currently have High Blood Pressure?

If the above condition(s) is/are present, answer questions 4a-4b — If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐

5. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes

If the above condition(s) is/are present, answer questions 5a-5e — If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

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6. Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome

If the above condition(s) is/are present, answer questions 6a-6b If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure

If the above condition(s) is/are present, answer questions 7a-7d If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia

If the above condition(s) is/are present, answer questions 8a-8c If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event

If the above condition(s) is/are present, answer questions 9a-9c If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. Do you have any other medical condition not listed above or do you have two or more medical conditions?

If you have other medical conditions, answer questions 10a-10c If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: _____

_____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

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If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact
www.eparmedx.com
Email: eparmedx@gmail.com

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11-01-2019

Appendix B: Consent Form

CONSENT FORM

The effects of branched-chain amino acid supplementation on muscle damage markers in recreational athletes

Informed Consent

I, _____ (please print), have read the Letter of Information / Consent document, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

Participant's name: _____ _____
(signature) (Print name here)

Date: _____

No legal rights are waived by signing.

Please indicate if you would like to be contacted for future studies by placing a check mark in the appropriate box below:

- □ I allow my contact information to be retained for contact about future studies.
- □ I do not allow my contact information to be retained for contact about future studies.

Person Obtaining Informed Consent: _____ _____
(Signature) (Print name here)

Date: _____

You will receive a copy of the consent form after it has been signed. You do not waive any legal rights by signing the consent form.

This letter is for you to keep for future reference.

Appendix C: Ethics Approval

Western Research

Date: 19 April 2019

To: Dr. Peter Lemon

Project ID: 113281

Study Title: The effects of branched-chain amino acid supplementation on muscle damage markers in recreational athletes

Application Type: HSREB Initial Application

Review Type: Full Board

Meeting Date: 29/Jan/2019 13:00

Date Approval Issued: 19/Apr/2019 10:59

REB Approval Expiry Date: 19/Apr/2020

Dear Dr. Peter Lemon

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above mentioned study as described in the WREM application form, as of the HSREB Initial Approval Date noted above. This research study is to be conducted by the investigator noted above. All other required institutional approvals must also be obtained prior to the conduct of the study.

Documents Approved:

Document Name	Document Type	Document Date	Document Version
BCAA proposal	Protocol	09/Jan/2019	1
DAta Collection Form - BCAA study March 15, 2019	Other Data Collection Instruments	15/Mar/2019	2
Exclusion Criteria Form version 3- BCAA Study clean copy	Other Data Collection Instruments	17/Apr/2019	3
Facebook Script for Recruitment -Crystal	Recruitment Materials	09/Jan/2019	1
Letter of Information - Crystal clean copy version 3	Written Consent/Assent	08/Apr/2019	3
PAR-Q+	Other Data Collection Instruments	03/Dec/2018	1
poster - crystal	Recruitment Materials	09/Jan/2019	1

Documents Acknowledged:

Document Name	Document Type	Document Date	Document Version
study design	Flow Diagram	09/Jan/2019	1

No deviations from, or changes to, the protocol or WREM application should be initiated without prior written approval of an appropriate amendment from Western HSREB , except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Nicola Geoghegan-Morphet, Ethics Officer on behalf of Dr. Philip Jones, HSREB Vice-Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix D: Pain Intake Questionnaire Package

Understanding Pain

Intake Questionnaire Package

Lead Researcher: David M. Walton PhD, Western University

ID No.: _____ Date: _____

General Characteristics

Are you:	◯ Male ◯ Female
Please indicate your age or year of birth:	Age: _____ OR Year of birth: _____
Please indicate your approximate:	Weight: _____ lbs or kg (circle) Height: ____ ft ____ inches **OR** _____ cm
Please indicate your highest level of education	☐ Did not finish high school ☐ High school ☐ Community college ☐ Professional / Trade school ☐ University undergraduate degree ☐ Master's Degree ☐ Doctorate ☐ Other (describe): _____
What is your average annual (combined) household income before tax?	☐ <$20,000 ☐ $20,000 - $40,000 ☐ $41,000 - $60,000 ☐ $61,000 - $80,000 ☐ $81,000 - $100,000 ☐ $101,000 - $150,000 ☐ $151,000 - $200,000 ☐ >$200,000
How many people live in your home?	_____

ID No.: _____ Date: _____

Please list the medications you are taking and their dosages

Name of medication	**Dose**	**Reason**
Example: ***Gabapentin***	***900 mg, 1/day***	***Neuropathic pain***

Please indicate if you are on a particular / special diet (e.g. Gluten-free, Paleo, Vegetarian, Vegan, Lactose-free, etc.) and whether this is due to medical or personal reasons

Diet Type:_____

Reason:_____

ID No.: _____ Date: _____

Are you currently taking any food supplements or vitamins, and how often do you take them?

Name of supplement	**Dose**
Example: ***Vitamin C***	***1000mg/day***
Example: ***Protein powder/shake***	***2 shakes (1L each) / day***

Do you participate in any hobbies or physical activity/sports, and how often do you participate?

Type of activity	**Frequency**
Example: ***Yoga***	***3 times a week***

ID No.: _____ Date: _____

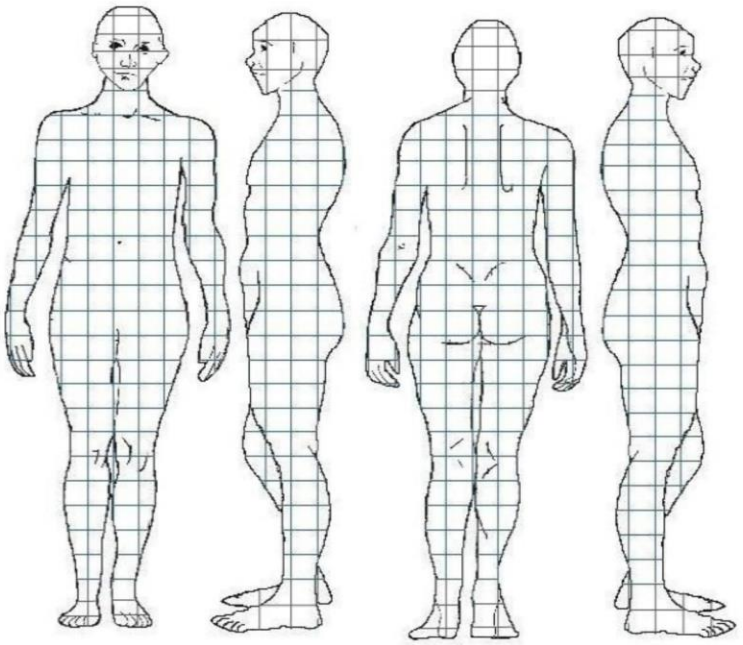
Please list any other chronic (long term) health conditions with which you have been formally diagnosed (e.g. type 2 diabetes, hypertension, Crohn's disease, high cholesterol, depression, etc…):

Condition (write additionally in the space below if required)
1.
2.
3.
4.
5.
6.
7.

ID No.: _____ Date: _____

On the diagrams below, please indicate the ares in which you are *currently* feeling symptoms.

1. First, **shade (colour)** the areas in which you are feeling pain.
2. Next, **circle** the areas in which you are feeling tingling, pricking or burning.
3. Finally, **place an 'N'** near the areas where you are feeling numbness, heaviness, or other sensations.

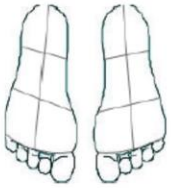


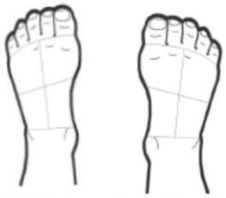

Palm of Left Hand


Palm of Right Hand


Back of Left Hand


Back of Right Hand


Sole of Left Foot Sole of Right Foot


Top of Left Foot Top of Right Foot

ID No.: _____ Date: _____

BPI

1. Please rate your pain by marking the box best describes your pain **at its worst** in the last 24 hours										
0	1	2	3	4	5	6	7	8	9	10
No Pain										Extreme Pain
2. Please rate your pain by marking the box beside the number that best describes your pain **at its least (best)** in the last 24 hours										
0	1	2	3	4	5	6	7	8	9	10
No Pain										Extreme Pain
3. Please rate your pain by marking the box beside the number that best describes your pain **on average**										
0	1	2	3	4	5	6	7	8	9	10
No Pain										Extreme Pain
4. Please rate your pain by marking the box beside the number that describes how much pain you have **right now**										
0	1	2	3	4	5	6	7	8	9	10
No Pain										Extreme Pain
5. Circle the number that describes how much, **during the past 24 hours**, pain has interfered with your:										
A. General Activity										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes
B. Mood										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes
C. Walking Ability										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes
D. Normal Work (includes both work outside the home and housework)										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes
E. Relations with other people										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes
F. Sleep										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes
G. Enjoyment of Life										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes

ID No.: _____ Date: _____

Gender, Pain and Expectations Scale

Gender: _____ Age: _____

1. How much do the following words describe you:

	Not At All	Very Little	Somewhat	A Lot	Extremely
Independent	□	□	□	□	□
Emotional	□	□	□	□	□
Gentle	□	□	□	□	□
Confident	□	□	□	□	□
Tough	□	□	□	□	□
Giving	□	□	□	□	□
Accepting	□	□	□	□	□
Leader	□	□	□	□	□
Competitive	□	□	□	□	□
Determined	□	□	□	□	□
Nurturing	□	□	□	□	□
Sensitive	□	□	□	□	□
Decisive	□	□	□	□	□
Patient	□	□	□	□	□

Here are 3 **different ways** people can be different about their pain.

1. **Pain sensitivity** is the amount of injury required to cause pain.
2. **Pain endurance** is how much time passes before a person needs help to manage their pain
3. **Willing to report pain** is how much people will tell others about their pain

How would you **rate yourself** on these aspects of pain?

	Not At All	Very Little	Somewhat	A Lot	Extremely
I am sensitive to pain	□	□	□	□	□
I can endure pain	□	□	□	□	□
I am willing to report my pain	□	□	□	□	□

Men or women can be **different** in these aspects of pain. Who do you think is more…

	Men-a lot more	Men-a little more	No difference	Women-a little more	Women- a lot more
…sensitive to pain	□	□	□	□	□
…able to endure pain	□	□	□	□	□
…willing to report my pain	□	□	□	□	□

How do well do you think you manage pain compared to other people of your gender?

□ Less well
□ The same
□ Better

Appendix E: Pain Follow-Up Questionnaire Package

Understanding Pain

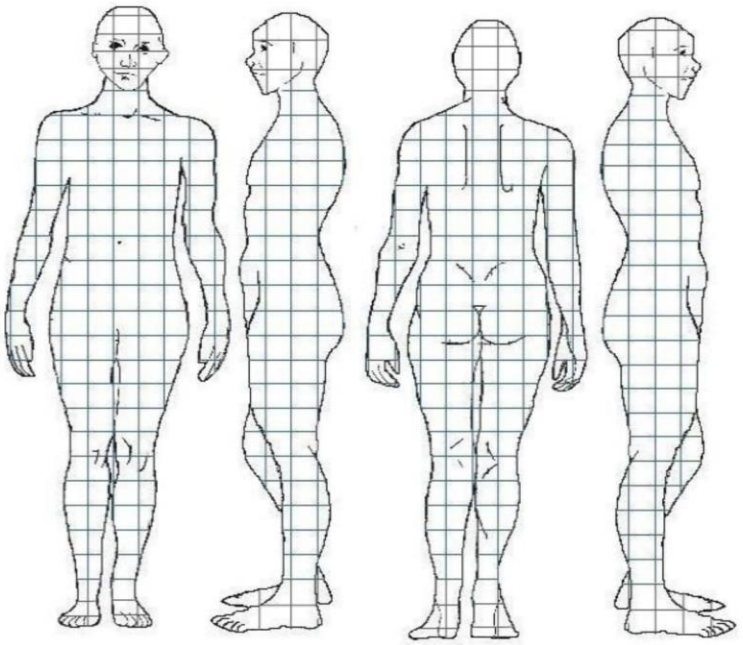
Follow-up Questionnaire Package

Lead Researcher: David M. Walton PhD, Western University

ID No.: _____ Date: _____

On the diagrams below, please indicate the ares in which you are *currently* feeling symptoms.

1. First, **shade (colour)** the areas in which you are feeling pain.
2. Next, **circle** the areas in which you are feeling tingling, pricking or burning.
3. Finally, **place an 'N'** near the areas where you are feeling numbness, heaviness, or other sensations.

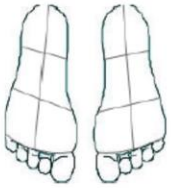


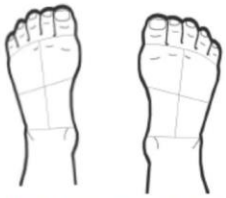

Palm of Left Hand


Palm of Right Hand


Back of Left Hand


Back of Right Hand


Sole of Left Foot Sole of Right Foot


Top of Left Foot Top of Right Foot

ID No.: _____ Date: _____

BPI

1. Please rate your pain by marking the box best describes your pain **at its worst** in the last 24 hours										
0	1	2	3	4	5	6	7	8	9	10
No Pain										Extreme Pain
2. Please rate your pain by marking the box beside the number that best describes your pain **at its least (best)** in the last 24 hours										
0	1	2	3	4	5	6	7	8	9	10
No Pain										Extreme Pain
3. Please rate your pain by marking the box beside the number that best describes your pain **on average**										
0	1	2	3	4	5	6	7	8	9	10
No Pain										Extreme Pain
4. Please rate your pain by marking the box beside the number that describes how much pain you have **right now**										
0	1	2	3	4	5	6	7	8	9	10
No Pain										Extreme Pain
5. Circle the number that describes how much, **during the past 24 hours**, pain has interfered with your:										
A. General Activity										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes
B. Mood										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes
C. Walking Ability										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes
D. Normal Work (includes both work outside the home and housework)										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes
E. Relations with other people										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes
F. Sleep										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes
G. Enjoyment of Life										
0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes

ID No.: _____ Date: _____

Patient-Rated Elbow Evaluation

*The questions below will help us understand the amount of difficulty you have had with your elbow in the past week. You will be describing your **average** elbow symptoms **over the past week** on a scale 0-10.*

1. PAIN

*Rate the average amount of pain in your elbow **over the past week** by circling the number that best describes your pain on a scale from 0-10. A **zero** (0) means that you **did not have any pain** and a **ten** (10) means that you had **the worst pain you have ever experienced.***

RATE YOUR PAIN:	
When it is at its worst	0 1 2 3 4 5 6 7 8 9 10
At rest	0 1 2 3 4 5 6 7 8 9 10
When lifting a heavy object	0 1 2 3 4 5 6 7 8 9 10
When doing a task with repeated elbow movement	0 1 2 3 4 5 6 7 8 9 10

How often do you have pain?	0 1 2 3 4 5 6 7 8 9 10 Never Always

ID No.: _____ Date: _____

2. FUNCTION

A. SPECIFIC ACTIVITIES

*Rate the **amount of difficulty** you experienced performing each of the items listed below, over the past week, by circling the number that best describes your difficulty on a scale of 0-10. A **zero** (0) means you did not experience any difficulty and a **ten** (10) means it was so difficult you were unable to do it at all.*

	No Difficulty										Unable To Do
Comb my hair	0	1	2	3	4	5	6	7	8	9	10
Eat with a fork or spoon	0	1	2	3	4	5	6	7	8	9	10
Pull a heavy object	0	1	2	3	4	5	6	7	8	9	10
Use my arm to rise from a chair	0	1	2	3	4	5	6	7	8	9	10
Carry a 10lb object with my arm at my side	0	1	2	3	4	5	6	7	8	9	10
Throw a small object, such as a tennis ball	0	1	2	3	4	5	6	7	8	9	10
Use a telephone	0	1	2	3	4	5	6	7	8	9	10
Do up buttons on the front of my shirt	0	1	2	3	4	5	6	7	8	9	10
Wash my opposite armpit	0	1	2	3	4	5	6	7	8	9	10
Tie my shoe	0	1	2	3	4	5	6	7	8	9	10
Turn the doorknob and open a door	0	1	2	3	4	5	6	7	8	9	10

B. USUAL ACTIVITIES

*Rate the **amount of difficulty** you experienced performing your **usual** activities in each of the areas listed below, over the past week, by circling the number that best describes your difficulty on a scale of 0-10. By "usual activities", we mean the activities that you performed **before** you started having a problem with your elbow. A **zero** (0) means you did not experience any difficulty and a **ten** (10) means it was so difficulty you were unable to do any of your usual activities.*

1. Personal activities (dressing, washing)	0	1	2	3	4	5	6	7	8	9	10
2. Household work (cleaning, maintenance)	0	1	2	3	4	5	6	7	8	9	10
3. Work (your job or everyday work)	0	1	2	3	4	5	6	7	8	9	10

ID No.: _____ Date: _____

4. Recreational activities	0 1 2 3 4 5 6 7 8 9 10

Comments:

Curriculum Vitae

Name:	Crystal Lee
Post-secondary Education and Degrees:	Brock University St. Catharines, Ontario, Canada 2014-2018, B.Sc. (Hons) The University of Western Ontario London, Ontario, Canada 2018-2020, M.Sc.
Honours and Awards:	Entrance Scholarship, Brock University, St. Catharines, Ontario**,** Canada 2014
Related Work Experience	Teaching Assistant The University of Western Ontario 2019-2020