THE EFFECT OF β-HYDROXY-β-METHYLBUTYRATE (HMB) SUPPLEMENTATION ON NEUROMUSCULAR PERFORMANCE FOLLOWING FATIGUING EXERCISE IN HEALTHY SUBJECTS

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THE EFFECT OF β-HYDROXY-β-METHYLBUTYRATE (HMB) SUPPLEMENTATION ON NEUROMUSCULAR PERFORMANCE FOLLOWING FATIGUING EXERCISE IN HEALTHY SUBJECTS

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DISSERTATION

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Kinesiology and Health Promotion at the University of Kentucky

By
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Lexington, Kentucky

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Lexington, Kentucky
2015
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ABSTRACT OF DISSERTATION

THE EFFECT OF $\beta$-HYDROXY-$\beta$-METHYLBUTYRATE (HMB) SUPPLEMENTATION ON NEUROMUSCULAR PERFORMANCE FOLLOWING FATIGUING EXERCISE IN HEALTHY SUBJECTS

Supporters of a nutritional supplement, $\beta$-Hydroxy-$\beta$-Methylbutyrate (HMB) supplementation, claim that it will increase the muscular strength gains and lean muscle mass gains seen during a resistance training program. It has been suggested that HMB supplementation does this by preventing muscle damage or by regenerating damaged muscle cell membranes. However, no research has evaluated the effect of HMB supplementation on low frequency fatigue. Therefore, the purpose of this study was to determine if three weeks of HMB supplementation could attenuate the effects of low frequency fatigue caused by eccentric muscle contractions of the tibialis anterior muscle. A total of 33 healthy recreationally active subjects (18 males, 15 females; 23.2 ± 4.3 yr) were recruited for this study. All subjects preformed 4 sets of 25 eccentric contractions of the tibialis anterior muscle through a range of motion of 30 degrees. Recovery measures were taken for 20 minutes after the fatigue protocol and at 48 and 96 hours of recovery. The recovery measures included: Maximum voluntary contraction peak torque, 10 Hz peak torque, 50 Hz peak torque, 10/50 Hz peak torque ratio, and EMG measurements. Each subject served as their own control and limbs were randomly assigned to pre-supplement or post-supplement limbs. Following the pre-supplement fatigue protocol and recovery measures each subject completed three weeks of 3g/day HMB supplementation. After the supplementation period the post-supplement fatigue protocol was completed and recovery measures were taken. The 10 Hz peak torque and the 10/50 Hz torque ratio in the pre-supplement limb was still significantly reduced at the 96-hour recovery measurement time, indicating that it was still showing low
frequency muscle fatigue at this time. Furthermore, the post-supplement limb, recovered from the fatigue protocol faster, and did not show any signs of low frequency muscle fatigue at the 48-hour recovery measurement time. In addition the pre-supplement limb had significant maximum voluntary contraction torque deficit at the 48-hour recovery measurement time and the post-supplement limb showed no significant deficits. The main findings of this study were that three weeks of HMB supplementation attenuated low frequency fatigue and maximum voluntary contraction torque reduction after an eccentric fatigue protocol.

Keywords: Eccentric contractions; low frequency fatigue; tibialis anterior; dorsiflexors; electrical stimulation

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TABLE OF CONTENTS

Acknowledgments...........................................................................................................................................iii
List of Tables.....................................................................................................................................................vi
List of Figures..................................................................................................................................................vii
Chapter I: Introduction.....................................................................................................................................1
  Background.....................................................................................................................................................1
  Statement of the Problem..............................................................................................................................2
  Purpose...........................................................................................................................................................3
  Hypotheses.....................................................................................................................................................3
  Significance...................................................................................................................................................3
  Delimitations................................................................................................................................................4
  Limitations...................................................................................................................................................4
  Assumptions................................................................................................................................................4
Chapter II: Literature Review..........................................................................................................................5
  Defining Fatigue............................................................................................................................................5
  Central Fatigue Versus Peripheral Fatigue.....................................................................................................5
  Mechanisms of Fatigue................................................................................................................................6
  Low-Frequency Fatigue Versus High-Frequency Fatigue...........................................................................8
  Eccentric Muscle Contractions and Damage................................................................................................11
  Rational of HMB Supplementation................................................................................................................13
  Mechanisms of Action of HMB......................................................................................................................15
  Research Studies Involving HMB Supplementation...................................................................................17
    HMB Used in Cancer and AIDS Research.................................................................................................18
  Research Studies That Reported No Effects of HMB Supplementation..................................................19
  Research Studies That Reported Positive Effects of HMB Supplementation.........................................24
  Conclusion....................................................................................................................................................30
Chapter III: Methodology..............................................................................................................................32
  Subjects.........................................................................................................................................................32
  Testing Procedures.......................................................................................................................................32
    Anthropometric and Body Composition Measurements............................................................................33
LIST OF TABLES

Table 2.1: Summary of Studies that Reported no Effects of HMB Supplementation.....23
Table 2.2: Summary of Studies that Reported Positive Effects of HMB Supplementation
………………………………………………………………………………………………………..29
Table 4.1: Subject Characteristic Measurements………………………………………………37
Table 4.2: Pre-Supplement and Post-Supplement Measures
for Fatigue Protocol and Recovery Period………………………………………………………43
Table 4.2 (Continued): Pre-Supplement and Post-Supplement Measures
for Fatigue Protocol and Recovery Period………………………………………………………44
LIST OF FIGURES

Figure 3.1: Study Overview ..............................................................................................................34
Figure 3.2: Pre and Post- Supplement Baseline and Fatigue Protocol Session Overview ........................................34
Figure 4.1: MVC Torque Measurements for Pre and Post-Supplement Limbs .................................................45
Figure 4.2: 10 Hz Peak Torque Measurements for Pre and Post-Supplement Limbs .........................................45
Figure 4.3: 50 Hz Peak Torque Measurements for Pre and Post-Supplement Limbs .........................................46
Figure 4.4: 10/50 Hz Torque Ratio for Pre and Post-Supplement Limbs .........................................................46
Figure 4.5: TA EMG for Pre and Post-Supplement Limbs ..............................................................................47
Chapter 1: Introduction

Background

When the human body participates in any strenuous physical activity there will be a decline in muscular performance during that activity and this decline is commonly called fatigue (2). Muscle fatigue can commonly be found in patients in hospitals, elderly people, and even middle aged people performing their activities of daily living (2). This type of fatigue is characterized by a loss of muscle strength that results from a decline in both muscular force and velocity of contraction. In addition fatigue is reversible by rest, and can be measured by a reduction in force, a change in electromyographic activity or an exhaustion of contractile function (11, 14).

Low frequency fatigue (LFF) is muscular fatigue that occurs at low frequencies of stimulation activation (34). Characteristics of LFF is the reduction in muscle force is decreased at low stimulation frequencies, recovery of force is slow, taking hours or days, and the effects remain in the absence of gross metabolic or electrical disturbance of the muscle (2, 3, 25, 35). This form of fatigue can be seen after any form of intense exercise and is a large contributor to the feeling of weakness in muscles after exercise (25, 84).

The primary implication of LFF is the reduction of muscle force at low frequency stimulation and is typically greater than 50% of rested values (25). Motor unit discharge rates during voluntary skeletal muscle activation rarely exceed 30 Hz, so any voluntary muscle contraction would be greatly affected by LFF (25). This would include any strenuous activity, walking, stair climbing, and all other activities of daily living (25).

It has been shown that one of the causes of LFF is the reduced amount of calcium (Ca²⁺) released from the sarcoplasmic reticulum (24). This reduces the amount of Ca²⁺ that can bind to troponin, reduces the amount of active muscle fibers, and therefore reduces the amount of force that the whole muscle can produce (25). A decrease in Ca²⁺ release affects muscle tension at low frequencies of stimulation to a much greater degree than muscle tension at high frequencies (25, 84). It seems likely that there is some degree of structural damage to one or more of the proteins or structures involved in excitation contraction coupling and the long time course of LFF represents repair or resynthesis of these proteins or structures (25, 84). In support of this muscle damage explanation it has been shown that LFF is a prominent characteristic following eccentric exercises and may be seen during the repair process if the muscle fiber was damaged during this type of contraction (25).
Eccentric muscle contractions produce more muscle damage than concentric or static contractions (2, 6, 61). These types of contractions are part of everyday normal activities such as, walking downstairs or lowering a heavy weight and the greater muscle damage that is caused is not related to metabolic fatigue but, it is due to a mechanical insult (6). In overstretched sarcomeres the sarcoplasmic reticulum could be damaged or its connection with the transverse tubules might be altered (2). The evidence for this is the LFF that is seen after eccentric muscle contractions (6). This LFF has been found to be caused by excitation contraction uncoupling and in particular a reduced amount of Ca\textsuperscript{2+} release from the sarcoplasmic reticulum (2, 6, 61, 83).

A nutritional supplement that has gained attention recently is β-Hydroxy-β-Methylbutyrate (HMB) and it claims to improve recovery following muscle damage. HMB has been associated with increased isometric, isokinetic, and dynamic strength and has been generalized across young, elderly, untrained, trained and clinically cachexic conditions (88). HMB is a metabolite of the essential branch chain amino acid leucine and is thought to act as an anticatabolic agent, minimizing protein breakdown and damage to cells which may occur with intense exercise (72, 79, 92). It has been suggested that HMB supplementation increases muscular strength and lean muscle mass gains, seen during a resistance training program, by preventing muscle damage or by regenerating damaged muscle cell membranes (26, 43, 46, 53, 66, 88).

**Statement of the Problem**

LFF can be seen after any form of activity and is a large contributor to the feeling of weakness in muscles after exercise and force reduction is typically greater than 50% of rested values (25, 84). In addition, recovery of force is slow, and can take hours or days (2, 3, 25, 35). Motor unit discharge rates during voluntary skeletal muscle activation rarely exceed 30 Hz, so any voluntary muscle contraction would be greatly affected by LFF (25). This would include any strenuous activity, walking, stair climbing, and all other activities of daily living (25).

HMB supplementation has been associated with increased isometric, isokinetic, and dynamic strength and has been generalized across young, elderly, untrained, trained and clinically cachexic conditions (88). HMB supplementation can potentially prevent muscle damage or enhance regeneration of damaged muscle cell membranes (26, 43, 46, 53, 66, 88). So, HMB supplementation may be effective in reducing LFF caused by
muscle damage and to this date a research study has not been found investigating the effects of HMB supplementation and the reduction of LFF. If supplementation with this substance can be shown to reduce LFF, caused by fatiguing physical activity, then it may provide a quicker recovery for recreational athletes, the general public, and people suffering from long term illnesses.

**Purpose**

The purpose of this study was to determine if three weeks of HMB supplementation could attenuate the effects of LFF caused by eccentric muscle contractions in young healthy adults.

**Hypotheses**

Hypothesis 1 – There will be two distinct types of fatigue after the eccentric fatigue protocol. This will include a high frequency fatigue, measured by the 50 Hz peak torque which will recover faster than the low frequency fatigue, measured by the 10 Hz peak torque.

Hypothesis 2 – HMB supplementation will reduce the recovery time of low frequency fatigue following eccentric exercise. The 10/50 Hz torque ratio and 10 Hz peak torque will recover toward baseline levels faster in the HMB supplemented limb.

Hypothesis 3 – HMB supplementation will reduce the recovery time of muscle damage caused by eccentric exercise. The MVC torque will recover toward baseline levels faster in the HMB supplemented limb.

**Significance**

The mechanisms of fatigue are complex and multiple mechanisms can influence muscle fatigue at the same time. Based on scientific studies it has been concluded that LFF is most likely caused by failure of excitation-contraction coupling or the reduction of cross bridge force production (32). There have been many hypotheses and theories on what causes LFF and over the years through different scientific experiments some of these hypotheses have been disproven. But, there has not been any single theory that has been proven to explain all of the variables involved in LFF nor have there been any procedures or substances that have been shown to entirely block or completely eliminate LFF. The contribution of this research is expected to be the first step in
discovering methods or substances that will reduce both LFF and muscle damage. In addition, this research will add to the literature of the severity and time course of LFF. This contribution will be significant because it will allow people to participate in their daily activities without decreased ability due to LFF and without the discomfort of muscle damage.

**Delimitations**

This study was delimited to recreationally active males and females between the ages of 18-35. “Recreationally active” was defined as a person who was physically active for 60 minutes per day, 3-5 days per week.

**Limitations**

A limitation of this study was that a direct measure of muscle damage was not used after the fatigue protocol. Another limitation of this study was that the subject’s physical activity and nutritional intake was not strictly controlled. The subjects were instructed to maintain their same nutritional intake and activity level during the supplementation and testing periods. In addition, a true control group or a placebo group was not used for comparison in this study.

**Assumptions**

It was assumed that all subjects gave a maximal effort during all testing procedures and that their nutritional intake and physical activity remained constant throughout the entire study.
Chapter II: Literature Review

Defining Fatigue

The term fatigue is very hard to define. First, we must distinguish between subjective fatigue and physiological fatigue. Subjective fatigue can be defined as physical or mental weariness resulting from exertion and it is a feeling that a person experiences (12). So, it is subjective and the amount of fatigue varies depending on the person being tested and is very hard to measure accurately and consistently. Physiological fatigue on the other hand is not subjective and is easier to measure. There are many different definitions of physiological fatigue but, for the purpose of this review it will be defined as the “failure to maintain the required or expected force” of a particular task (32). This type of fatigue is characterized by a loss of muscle strength that results from a decline in both muscular force and velocity of contraction, and is reversible by rest (14). Muscular fatigue can be measured by a reduction in muscle force, a change in electromyographic activity or an exhaustion of contractile function (11).

Another problem with defining physiological fatigue is the question: when does fatigue occur? It is accepted that fatigue is not the point of task failure or when muscles become exhausted (11). But, it is a decrease in maximal force that the muscles can produce and it develops soon after the onset of physical exercise (11).

Central Fatigue Versus Peripheral Fatigue

Physiological fatigue can be broken down into two components: central fatigue and peripheral fatigue (32). Central fatigue is impaired muscular performance that arises from the central nervous system (12). This type of fatigue may be affected during voluntary exercise by emotions or other psychological factors (32). Central fatigue may also be affected by descending motor pathways, interneurons, and motoneurons in the central nervous system (brainstem and spinal cord) (32). A major problem in measuring central fatigue is that very little is known about the neurons involved in the generation of volitional effort (32). For this reason most of the experiments designed to test muscular fatigue aim to limit the effects of central fatigue, by electrically stimulating muscles rather than having the subject perform volitional contractions.
The second component of physiological fatigue is peripheral fatigue (32). This type of fatigue occurs when one or several of the physiological processes that enable the contractile proteins to generate force become impaired or reduced (11). Peripheral fatigue occurs outside of the central nervous system and can be caused by reduced impulse conduction in the motor axons and their terminals, reduced neuromuscular transmission to the muscle, reduced conduction of impulses in the muscle fibers, reduced excitation-contraction coupling (E-C coupling), or impairments in the contractile process itself (11). There is no single cause of peripheral muscle fatigue, it can result from multiple factors acting at various sites, and a combination of factors can be involved (14). For the duration of this review the term fatigue will imply physiological peripheral fatigue.

There are several methods that are used to induce and measure muscle fatigue. A very common technique is to stimulate the muscle with an external electrical stimulus (32). A muscle twitch is developed by a short burst of electrical activity and has been used to evaluate post-fatigued whole muscles and muscle fibers (14). Some features that can be seen in a post-fatigued muscle fiber or whole muscle are: reduced twitch force, reduced whole muscle force, prolonged contraction and half-relaxation times, and reductions in the peak rate of tension development (14). These changes in muscle fibers will lead to a decline in muscular performance and will include reduced force production, decreased velocity of muscle shortening, and slowed muscle relaxation (2).

Mechanisms of Fatigue

Before the mechanisms of fatigue are discussed a review of the events that occur to cause muscle contraction will be given. This process starts in the central nervous system that sends an action potential down the α-motoneuron (86). The action potential travels down the motoneurons and reaches the nerve axon terminal, at this time voltage-gated Ca²⁺ channels open and Ca²⁺ enters the axon terminal (86) . This causes vesicles to migrate to the axon terminal end bud and release the neurotransmitter acetylcholine into the synaptic cleft (86) . Acetylcholine reaches the end plate of the muscle fiber, binds with postsynaptic receptors, and causes a local depolarization (86). This depolarization causes an action potential on the muscle fiber by activating voltage-gated sodium and potassium channels (86). Next, acetylcholine is
removed from the synaptic cleft and the action potential is propagated along the surface of the muscle fiber (86). At this point the process of excitation-contraction coupling (E-C coupling) begins. As an action potential is propagated down the muscle fiber the first step of E-C coupling is the action potential moves into the interior of the muscle fiber through the T-tubules (32). This causes the voltage sensors, the dihydropyridine receptors (DHPR), to signal the ryanodine receptors (RYRs) to open (32). When the RYRs are open a rapid escape of calcium (Ca$^{2+}$) from the sarcoplasmic reticulum (SR) enters the cytosol of the muscle fiber (32). Some of the Ca$^{2+}$ in the muscle fiber cytosol combines with troponin on the actin filaments which starts the cross bridge cycle that produces muscle shortening and force (32). Once the action potential has ended, Ca$^{2+}$ is pumped back into the SR by a membrane-bound Ca$^{2+}$ ATPase and breaks the Ca$^{2+}$-troponin complex which stops the cross bridge cycle (32).

Any abnormalities in any of the process just described have the possibility to contribute to muscle fatigue. The mechanisms of fatigue are complex and multiple mechanisms can affect muscle fatigue at the same time. Based on scientific studies it has been concluded that muscle fatigue is most likely caused by failure of E-C coupling or the reduction of cross bridge force production (32).

The first way that fatigue can affect muscle force during E-C coupling is by reducing the amount of Ca$^{2+}$ that is released from the RYR into the muscle fiber cytosol (32). If a smaller amount of Ca$^{2+}$ is released, then a smaller number of cross bridges will be activated. It has been shown that the concentration of inorganic phosphate (P$_i$) increases in the muscle fiber during muscle contractions (2). This is because the breakdown of phosphocreatine (PCr) to creatine (Cr) and P$_i$ is essential to produce ATP rapidly for muscle contraction (2). It has also been shown that an increase in P$_i$ in the muscle fiber reduces Ca$^{2+}$ release and therefore reduces cross bridge cycling and force production (2). This type of muscle fatigue can be reversed by agents such as caffeine, which opens the RYR and allows a greater amount of Ca$^{2+}$ to enter the muscle fiber (2).

Another way that the increased P$_i$ concentration can influence muscle force is by slowing down the rate of the membrane-bound Ca$^{2+}$ ATPase (2). This will slow down the removal of Ca$^{2+}$ from the muscle fiber back into the SR (2). This will in turn slow down the relaxation rate of the cross bridge cycles and can delay the next cycle which may contribute to muscle fatigue (2).
Fatigue can also affect E-C coupling by a reduction in Ca\textsuperscript{2+} sensitivity (32). Reduced Ca\textsuperscript{2+} sensitivity is a condition where force at a given free Ca\textsuperscript{2+} concentration is lowered (32). So, in this case it is not the Ca\textsuperscript{2+} release that is the problem but, it is the fact that not as much force is produced from the given Ca\textsuperscript{2+} release. In other words it would take more Ca\textsuperscript{2+} release for the same amount of force production. The accumulation of P\textsubscript{i} is also thought to reduce Ca\textsuperscript{2+} sensitivity in a fatiguing muscle (2).

Another area that fatigue can influence force production is during the cross bridge cycle (14). It has been shown that there is a slower dissociation of actin from myosin in fatigued muscle fibers (14). This would slow down the rate of contraction of the muscle fiber and therefore reduce the force production. This slowing of cross bridge cycling is thought to be caused by an increase in hydrogen ions from the production of lactic acid by the contracting muscles, and by the increase of P\textsubscript{i} (14).

If a muscle fiber does not have ATP then the myosin heads cannot detach from the actin molecule and rigor development can set in (84). This of course does not happen in human muscle in vivo (84). So, it is thought that there may be a safety mechanism built into muscles that reduces Ca\textsuperscript{2+} release when energy consumption can no longer be met, to reduce the amount of ATP being consumed and protect the muscle from going into rigor (84). This of course will also decrease the amount of force produced and increase the amount of fatigue in the muscle.

There have been many hypotheses and theories on what causes muscle fatigue. Over the years through different scientific experiments some of these hypotheses have been disproven. But, there has not been one theory that has been proven to explain all of the variables involved in muscle fatigue. There is a way to produce muscle fatigue by sub maximal contractions that does not change any metabolite concentration a great deal (32). Based on this and the close correspondence between force and intracellular Ca\textsuperscript{2+} concentration, it has been determined that the likely cause of muscle fatigue occurs somewhere in the E-C coupling phase of muscle contraction (32).

**Low-Frequency Fatigue Versus High-Frequency Fatigue**

Peripheral physiological muscle fatigue can also be sub grouped into two different categories based on their physiological mechanisms (35, 37). These two groups are low frequency fatigue at 5 to 30 Hz and high frequency fatigue at 50 to 100 Hz (35). High frequency fatigue is induced by high frequency stimulation and is
characterized by rapidly recovering loss of force at high frequencies (37). On the other hand, low frequency fatigue is induced by low frequency stimulation and characterized by long term loss of force at low frequencies (37). High frequency fatigue recovers very quickly usually within minutes after stimulation or exercise has stopped (35). Based on the fast recovery of this type of fatigue the mechanisms are thought to be impaired muscle fiber membrane excitation, which is associated with an accumulation of potassium in the inter-fiber space or in the extracellular fluid contained in the transverse tubular system (35).

The second type of peripheral physiological muscle fatigue is low frequency fatigue (LFF). The characteristics of LFF are that muscle force is decreased most severely at low stimulation frequencies, recovery of force is slow, taking hours or days, and the effects remain in the absence of gross metabolic or electrical disturbance of the muscle (2, 3, 25, 35). This form of fatigue can be seen after any form of intense exercise but, is more pronounced after eccentric contractions, and is a large contributor to the feeling of weakness in muscles after exercise (25, 84). Since eccentric contractions cause LFF and its very slow recovery, it is possible that it is a consequence of some form of damage caused by the generation of high forces within the muscle (22). Also, LFF has been induced by voluntary contractions, high frequency stimulation (100 Hz), and low frequency stimulation (10-40 Hz) (25). This type of fatigue is typically measured by recording torque response to different frequencies of electrical stimulation (25). The change in the ratio of force production with 10-20 Hz stimulation compared to the force production with 80-100 Hz stimulation is a fatigue index of LFF that is commonly used (25). Any decrease in this ratio, compared before and after exposure to a fatiguing protocol, is interpreted as LFF (25).

The primary implication of LFF is the reduction of muscle force at low frequency stimulation (25). Observed force decrements with LFF in human skeletal muscles are typically greater than 50% of rested values (25). Motor unit discharge rates during voluntary skeletal muscle activation rarely exceed 30 Hz, so any voluntary muscle contraction would be greatly affected by LFF (25). This would include any strenuous activity, walking, stair climbing, and all other activities of daily living (25). This effect of LFF on voluntary muscle contraction could result in higher activation of the central nervous system, require the recruitment of a larger number of motor units or increased
firing frequency, and cause greater feelings of effort during repetitive activities and may limit performance (25).

It is clear that the mechanisms of LFF is caused by disruption within the muscle rather than in the nervous system because, during LFF the muscle electromyogram appears normal and it has been shown to occur in isolated muscle fibers (2). Also, LFF may not have a ionic or metabolic basis like other types of fast recovery fatigue because it takes so long to subside (25). There is evidence that LFF is caused by a defect in E-C coupling due to the fact that nearly maximal muscle tension can be achieved by high frequency stimulus or the addition of caffeine (2). The point in E-C coupling that LFF may affect is not known but, possible events include: failure of Ca^{2+} release from SR, reduced Ca^{2+} sensitivity of the contractile proteins, reduced conduction of the action potential in the T-tubules, and reduced Ca^{2+} reuptake by the SR (25, 84).

Westerblad et al. have demonstrated in mouse single muscle fibers that Ca^{2+} sensitivity of the contractile proteins is unchanged and Ca^{2+} release from the SR is reduced at all stimulus frequencies during LFF (84). They also reported that Ca^{2+} release was uniform across the entire muscle fiber, suggesting the T-tubule conduction remains adequate (84). In this experiment Ca^{2+} reuptake was moderately reduced, but it was unlikely to be the cause of the reduced amount of Ca^{2+} in the muscle fiber (84). The authors concluded that LFF is caused by a reduced Ca^{2+} release from the SR (84). This reduced amount of Ca^{2+} from the SR reduces the amount of Ca^{2+} that can bind to troponin, reduces the amount of active muscle fibers, and therefore reduces the amount of force that the whole muscle can produce (25). The reduced Ca^{2+} release could be caused by: failure of the action potential in the surface membrane, failure of T-tubule conduction, abnormal function of DHPR in the T-tubules, or abnormal function of the RYRs (85). Due to the long time course of recovery of LFF, which suggests that the reduced Ca^{2+} release may last longer than 24 hours, it is likely that a structural change involved in E-C coupling occurs and the long recovery time represents repair or resynthesis of this change (85).

A decrease in Ca^{2+} release affects muscle tension at low frequencies of stimulation to a much greater degree than muscle tension at high frequencies (25, 84). This can be seen when the concentration of intracellular Ca^{2+} release is plotted against the muscle tension produced (25). In this graph it can be seen that the Ca^{2+} release at high frequency stimulation occurs at the plateau of the Ca^{2+}-tension curve so, a change
in the intracellular Ca$^{2+}$ concentration would not yield a large change in the amount of tension produced by the muscle (25, 84). On the other hand, low frequency stimulation occurs on the steep portion of the Ca$^{2+}$-tension curve and a small change in the intracellular Ca$^{2+}$ concentration would yield a large change in the muscle tension produced (25, 84).

It has been shown that the most likely cause of LFF is reduced Ca$^{2+}$ release from the SR (2). There are many possibilities for the cause of this reduced Ca$^{2+}$ release and the most important evidence that needs to be considered is the long time course of LFF, which suggests that reduced Ca$^{2+}$ release can last for longer than 24 hours (25, 84). With this in mind it seems likely that there is some degree of structural damage to one or more of the proteins or structures involved in E-C coupling and the long time course of LFF represents repair or resynthesis of these proteins or structures (25, 84). In support of this muscle damage explanation it has been shown that LFF is a prominent characteristic following eccentric exercises and may be seen during the repair process, if the muscle fiber was damaged during this type of contraction (25).

**Eccentric Muscle Contractions and Damage**

Eccentric muscle contractions are lengthening muscle contractions or stretching of contracting muscles and they occur when the imposed load is greater than the muscle force generated (2, 45). Eccentric contractions produce more muscle damage than concentric or static contractions (2, 6, 61). These types of contractions are part of everyday normal activities such as, walking downstairs or lowering a heavy weight (2). Just walking requires muscles to undergo repetitive cycles of concentric and eccentric muscle actions (68). In general any movement of a joint that is controlled by an agonist and antagonist muscle pair, one will be contracting eccentrically and the other will be contracting concentrically (2). In physical activity if there is a large eccentric component to the activity, weakness will be apparent during and immediately after the activity but, pain, tenderness, swelling and stiffness develop more slowly and last much longer (2). These latter symptoms are most prominent on the first or second days after the physical activity and they are termed delayed onset muscle soreness (DOMS) (2). DOMS is thought to start with an initial mechanical event, leading to microscopic disruptions within the muscle fibers, which then lead to cell membrane damage and the loss of intracellular Ca$^{2+}$ homeostasis (4). The functional implications of this type of damage caused by eccentric muscle contractions are significant and include a decreased joint range of
motion, altered fatigability, muscle soreness, and decreased muscle shortening velocity, but it is the prolonged strength loss that is the most widely recognized as the largest restriction to physical activity (83).

After a bout of eccentric exercise muscle soreness appears 24 hours after exercise and peaks at 2-3 days post-exercise (9). Soreness slowly dissipates and does not fully subside until 8-10 days after exercise (9). Swelling and edema have also been suggested to cause soreness which is likely due to an increase in muscle pressure (9). Muscle strength loss is dramatic immediately after eccentric muscle contractions, recovers slowly, and by 10 days after exercise a deficit still remains (9). This strength loss may be caused by damaged myofibrils, a change in neural activation patterns, or sarcomeres stretched out by the performance of the lengthening actions (9). Sarcomere length is not uniform over the length of the fiber because, shorter sarcomeres are found toward the ends (9). If the lengthening actions pull some of the central sarcomeres apart, the overlap between the actin and myosin filaments would be reduced, which would reduce the maximal number of cross bridges, and reduce the amount of force production (9). Another reason that force is reduced after eccentric contractions could be the decline of $\text{Ca}^{2+}$ release from the SR (9). If the SR is damaged due to the lengthening contractions $\text{Ca}^{2+}$ release would be reduced and the ability to generate normal force levels would be impaired (9). Immediately after eccentric exercise range of motion is decreased and the ability to fully contract the muscle is decreased and is not restored until 10 days after the exercise (9). This could be because of the loss in muscle strength and could be related to the overstretched sarcomeres or the decreased $\text{Ca}^{2+}$ release from the SR (9).

Exercise protocols that use concentric muscle actions typically produce strength losses of 10-30% immediately after exercise, with strength returning to baseline within hours after exercise (8). Also, this type of muscle action does not produce damage and since there is a fast restoration of force loss, the fatigue is generally considered to be due to metabolic or neural factors (7). On the other hand high force eccentric exercise protocols can often generate up to 50-65% loss of force generating capacity when compared with pre-exercise values (8). Prolonged force loss after this type of muscle contractions can typically last one to two weeks and is thought to be caused by initial damage during the exercise and additional damage during the regeneration process (8).
Maximal eccentric muscle contractions can produce greater output forces than concentric and isometric muscle contractions but, they produce lower electromyographic (EMG) activity (6). The amount of integrated electromyographic (IEMG) activity during eccentric contractions have been shown to be approximately half that recorded during concentric contractions at similar workloads (38). If the IEMG is taken to reflect the total amount of muscle fiber activity, then during eccentric contractions about half the number of fibers are being activated to generate a given tension when compared to concentric contractions (38). Muscle damage caused by eccentric muscle contractions has been documented directly by muscle biopsy samples and indirectly from losses in strength and range of motion, increases in muscle soreness, and increases in muscle proteins, such as creatine kinase (CK) and lactate dehydrogenase (LDH), in the blood as markers for damage (64). Among these markers, change in force generating capacity is considered to be the most reliable. As a muscle lengthens, the ability to generate tension increases and a higher load is distributed among the same number of fibers, resulting in a higher load per fiber ratio (6). Also, since during eccentric contractions there are less active muscle fibers, the load per fiber ratio is increased when compared to concentric contractions (11). The fact that soreness and swelling peaks 24-48 hours post-exercise suggests that the initial damage may be exacerbated in the days after exercise (6). Inflammation occurring after the initial insult is likely responsible for the continued damage and may also function in the regeneration process (6).

Rational of HMB Supplementation

There have been many different methods and nutritional supplements that have been used to attempt to reduce the effects of fatigue, increase fat free mass and strength, and improve muscular performance, in an attempt to increase the results of physical exercise. Recreational athletes and health conscious people are looking for a way to get more out of their workout sessions to improve their body image or to increase their physical fitness level (54). Billions of dollars are spent each year on nutritional supplements and most of these supplements claim to reduce fat mass, increase fat free mass, or help to increase muscular strength (58). The most popular performance enhancing supplements are protein and amino acids because they are essential for the synthesis of structural proteins and are involved in numerous metabolic pathways that are associated with exercise (58). In particular, a great deal of attention has been paid
to the three branched-chain amino acids, leucine, isoleucine, and valine (19). To enhance the increase of muscle mass in the human body in response to an exercise stimulus there has to be either an increase in protein synthesis or a decrease in protein catabolism (73). Acute changes in amino acid metabolism caused by exercise are largely catabolic with a net negative balance between the rates of protein synthesis and protein breakdown (44). After exercise skeletal muscle remains in negative protein balance until adequate protein and energy are available for recovery (44). This is the reason why supplementation with protein and amino acids are very popular with people who are active and who are trying to increase their gains from their workout programs. Leucine is one of the most popular amino acids that people supplement with because of its roles in protein metabolism, glucose homeostasis, insulin action, and recovery from exercise (87).

During leucine metabolism, it is first transaminated to its ketoacid, α-ketoisocaproate (KIC) (78). At this point most of the KIC enters the mitochondria and is oxidatively decarboxylated to isovaleryl-CoA by branched-chain α-ketoacid dehydrogenase (78). But, a small amount of KIC (approximately 5%) is converted to β-hydroxy-β-methylbutyrate (HMB) by KIC dioxygenase in the cytosol of the liver (Figure 2.4 – left side) (78). In total only approximately 2 to 10% of leucine oxidation proceeds to HMB (13, 73). HMB is then converted to HMG-CoA which is converted to mevalonate and finally cholesterol by the enzyme HMG-CoA reductase (42).

In vitro leucine and KIC are both proposed to decrease nitrogen and protein loss by inhibiting protein breakdown (40). Also, leucine has been shown to stimulate protein synthesis and inhibit protein degradation in skeletal muscle of intact rats (19). But, animal and human studies have not shown an anabolic effect except in situations of either severe stress or trauma in which proteolysis is greatly elevated (40). This suggests that KIC and leucine are active only during periods of excessive catabolism or that a further metabolic product of leucine and KIC may be responsible for the anticatabolic effects of these compounds (40). HMB has recently gained attention as a nutritional supplement and it claims to increase muscle strength, muscle size, and fat oxidation (58, 70, 73, 77, 80, 88). It has also been associated with increased isometric, isokinetic, and dynamic strength and has been generalized across young, elderly, untrained, trained and clinically cachexic conditions (88).
HMB is a metabolite of the essential branch chain amino acid leucine and is thought to act as an anticatabolic agent, minimizing protein breakdown and damage to cells which may occur with intense exercise (17, 21, 26, 46, 49, 50, 53, 55, 57, 58, 73, 80, 91). HMB is present in both plants and animal foods such as catfish and citrus fruits in small amounts (0.3-0.4 g/d) are produced endogenously (13, 18, 54). Although HMB can be synthesized endogenously from leucine, it would take approximately 60g of leucine to reach an HMB dosage of 3 g/d, which is the recommended dosage for HMB supplementation (13). To obtain 60g of leucine from the diet, one would have to consume at least 600g of protein from high leucine containing foods such as eggs, dairy, and meats (13). Since this is not practical, to reach higher levels of HMB in the body requires nutritional supplementation (13). When supplemental HMB is consumed, HMB peaks in circulation at one to two hours after supplementation and reaches baseline levels by nine hours (13). Also, when HMB is supplemented at 1 to 6 g/d it was shown that approximately 14 to 29% was excreted in the urine so, it appears that a majority of supplemental HMB remains in the body (13).

HMB supplementation has been suggested to impart anticatabolic properties and to reduce muscle damage based on reduced enzyme markers of muscle damage such as creatine phosphokinase (CK), lactate dehydrogenase (LDH), and 3-methylhistidine (3-MH) (17, 21, 55, 70). Based on this HMB has been hypothesized to be the metabolic product of leucine and KIC that is responsible for the anticatabolic effects and to facilitate an increase in muscle mass and strength gains when combined with resistance training (53, 58).

**Mechanisms of Action of HMB**

The major claims that are made by promoters of HMB supplementation are that it will increase the muscular strength gains and lean muscle mass gains seen during a resistance training program (40). It has been suggested that HMB supplementation does this by preventing muscle damage or by regenerating damaged muscle cell membranes (26, 43, 46, 53, 66, 88). This anticatabolic effect has been suggested to be achieved by two mechanisms (26, 40, 50). The first mechanism involves conversion of HMB, in the cytosol of the liver, to β-hydroxy-β-methylglutarate-CoA (HMG-CoA) (26, 40, 50). HMG-CoA is then employed in cholesterol synthesis and could be rate limiting when cholesterol synthesis is in high demand, such as during periods of rapid cell growth or membrane repair that is seen with resistance training (17). Without
cholesterol, cells cannot experience normal growth so, most cells are capable of making their own cholesterol (90). But, there are many steps involved in cholesterol biosynthesis and much valuable cellular energy is utilized in the complex pathway to produce cholesterol (90). Also, stressed or damaged muscle cells may not be able to make sufficient HMG-CoA to support adequate cholesterol synthesis for cell functions (41). Supplemental HMB therefore, may be a source of HMG-CoA in damaged muscle cells to maintain adequate cholesterol synthesis and plasma membrane function (41). It has also been proposed that HMB supplementation provides a saturating source of cytosolic HMG-CoA for cholesterol synthesis and, in turn, allows for maximal cell growth and function (72). This increase in cholesterol synthesis has not been shown to increase blood lipid profiles (41). In fact, it has been shown that 3 to 8 weeks of HMB supplementation decreased total cholesterol by 3.7% and LDL cholesterol by 5.7% (41).

The second mechanism is that HMB is polymerized and covalently binds with cell membrane structures to enhance membrane integrity (49, 50). This increased integrity may also be accomplished through the additional source of cholesterol provided by HMB supplementation (46). It has been hypothesized that the de novo synthesized cholesterol is used to support the cell membrane integrity and help prevent muscle damage or used to regenerate damaged muscle cell membranes (26). Thus, HMB may provide the necessary amount of HMG-CoA for cholesterol synthesis and membrane protection that would then result in decreased muscle damage and quicker recovery from resistance exercise (17). This is supported by studies that have demonstrated that HMB supplementation has decreased markers of muscle damage following strenuous exercise (17, 26, 40, 55). This could also be one reason that HMB supplementation does not produce strength gains in subjects that are highly trained with resistance exercises (66). This is because with resistance training protein turnover and resistance exercise induced muscle damage are attenuated and HMB supplementation may not be able to reduce them further (66). Also, local deficiencies in muscle cell cholesterol are thought to occur due to cell hypertrophy (54). This could cause cell growth to decrease due to inadequate cholesterol available for cell membrane synthesis (54). Therefore, a larger supply of cholesterol for cell membrane synthesis, due to HMB supplementation, could lead to further muscle hypertrophy and larger lean muscle gains (54).

A more recent review has postulated that HMB supplementation could have other mechanisms (91). These mechanisms include an up regulation of IGF-I gene expression in skeletal muscles, stimulation of protein synthesis by increasing the mTOR
signaling pathway, and suppression of proteolysis by the inhibition of the UPP system (91). It is established that IGF-I exerts an anabolic action in skeletal muscle, leading to hypertrophy of muscle fibers (91). It has been observed that muscle IGF-I expression was increased after a culture of myoblasts were exposed to HMB (13). It has also been reported that IGF-I expression has been increased in whole animal models with HMB supplementation (28, 57). The enzyme mTOR (mammalian target of rapamycin) is a protein kinase that is involved in cell growth and HMB appears to act upon the mTOR pathway but the mechanisms are not yet known (13, 91). HMB supplementation may attenuate muscle protein losses through reduction of the UPP (91). The ubiquitin-proteasome system is up regulated in catabolic states and HMB has been shown to decrease the activity of this pathway, thereby possibly reducing protein degradation (13, 91). Also, changes in the rates of protein degradation rather than changes in rates of protein synthesis are the principle means for altering protein levels in many physiological and pathological conditions (73). These three possible mechanisms of HMB supplementation have not been fully researched yet. Most of these studies have been completed in animal models or single muscle fibers (13, 28). Further research is needed into how HMB supplementation may affect muscle growth and protein degradation through these three mechanisms.

Research Studies Involving HMB Supplementation

A meta-analysis conducted by Nissen and Sharp in 2003 reported that out of about 250 substances marketed as dietary supplements, only creatine and HMB were found to be effective in augmenting lean tissue gains with resistance training (43). In a second meta-analysis conducted by Rowlands and Thomson it was reported that HMB supplementation caused beneficial increases in lower body and overall average strength in untrained lifters, and strength was not affected by HMB in trained lifters (65). In a third review article Wilson et al. reported that many studies support the efficacy of HMB as an effective ergogenic aid for athletes to decreases DOMS, markers of muscle damage, and body fat, while increasing various markers of performance, including lean body mass and strength in resistance trained athletes (87). Also, there were a number of studies analyzed that did not support the efficiency of HMB supplementation (87). The authors suggested that the conflicting results may in part be attributed to the variability in humans, inadequate sample sizes, and methodological issues such as the specificity of
testing conditions, cases of overtraining, elicitation of an inadequate training stimulus in experienced participants, limited dependent variables, and short duration experiments (87). But, the results of HBM supplementation does not yield any clear cut conclusions. There have been human studies that have reported reduced muscle damage and upper body strength gains in both previously untrained men and women (17, 40, 55, 80). There have also been studies that have reported increases in muscle mass, muscle strength, reduced muscle damage, and anaerobic properties in previously trained men and women (26, 58, 77). HMB supplementation has also been shown to positively affect strength, fat free mass, protein synthesis, and functionality in elderly men and women (15, 82).

Vukovich et al. conducted a study to investigate the kinetics of HMB supplementation (81). Plasma HMB peaked at about 120 nmol/L within 2.0 ± 0.4 hours after consumption of one gram HMB and peaked at about 480 nmol/L within 1.0 ± 0.1 for three grams of HMB consumed (81). These values remained stable for the next 90 minutes, at which there was a gradual decline until 9 hours after consumption at which time values were not significantly different from baseline (81). The percent accumulation of HMB in the urine reached a level of about 14% of the given dose following the consumption of one gram of HMB and about 29% for a dose of three grams of HMB (81). The authors concluded that with HMB supplementation, plasma HMB levels peak between one and two hours and a small amount of HMB is excreted in the urine with the remaining being retained in the body for further metabolism (81).

HMB Used in Cancer and AIDS Research

Patients with cancer induced weight loss show a depression of protein synthesis in skeletal muscle and an increase in protein degradation, via the UPP pathway (74). The cachexia syndrome is seen in a variety of conditions including: cancer, surgery, trauma, sepsis, and AIDS (47). It is characterized by anorexia, weight loss, weakness, anemia, edema, and skeletal muscle wasting (47). Cancer cachexia has been cited as the most frequent cause of morbidity and mortality in patients with cancer and maintenance of lean tissue mass should lead to improved survival and decreased morbidity (36). Anabolic stimuli or an increase in calorie intake has been shown not to increase lean body weight in cachectic patients which suggests that protein degradation must be attenuated before muscle mass can increase (74). Recent studies have indicated that HMB was capable of attenuating protein degradation in skeletal muscle of
 cachectic mice and also stimulated protein synthesis resulting in an increase in the nonfat carcass mass (47, 74). The reduction in protein degradation was found to be caused by down-regulating the increased expression of the UPP pathway in the cachectic mice (74).

A study conducted by May et al. tested a combination of HMB, L-glutamine, and L-arginine supplementation in cancer patients who demonstrated a weight loss of at least 5% (36). It was reported that this combination increased both body mass and fat free mass relative to the control group (36). Also, these changes were apparent as early as 4 weeks after the start of supplementation (36). The authors concluded that this combination likely provided the substrates necessary for the body to minimize wasting and replenish lean body mass (36).

Just like in cancer patients, AIDS patients show a disproportionate loss of lean tissue with a preservation of fat mass (5). Also, these patients consume adequate amino acids and energy to support muscle maximal protein synthesis so, the problem of wasting is probably due to an accelerated rate of muscle catabolism relative to protein synthesis (5). A study conducted by Clark et al. showed through air displacement plethysmography, computerized tomography of leg muscles, and measurements of midarm circumferences that a combination of HMB, L-glutamine, and L-arginine increased lean tissue in AIDS-wasted patients (5). The authors concluded that this mixture favorably altered the rates of protein breakdown relative to protein synthesis and resulted in net protein accumulation (5).

The loss of muscle mass in cancer cachexia appears to be caused by a tumor product, proteolysis-inducing factor (PIF), which increases the UPP protein degradation pathway (75). In two separate studies it was reported that HMB was an effective agent for the management of muscle wasting in cancer induced protein degradation in murine myotubes (10, 75). It was concluded in both studies that HMB produced these positive effects by attenuation of PIF-induced protein degradation mediated through the UPP pathway (10, 75).

Research Studies That Reported No Effects of HMB Supplementation

Not all research studies have shown favorable results associated with HMB supplementation. In general authors have reported that HMB supplementation did not have an effect on muscular strength, muscular endurance, or body composition in
trained males (49, 50, 70). Also, short supplementation of HMB (less than 14 days) did not show any benefit in recovery of muscle function after exercise induced muscle damage or any other ergogenic responses in previously trained or untrained males (21, 46, 53).

There were five research studies found that used trained subjects and reported no effects of HMB supplementation (Table 2.1). The first of these studies was conducted by Ransone et al. and used Division I college football players as subjects (62). The subjects in this study supplemented with 3g per day of HMB for 28 days (62). During the supplementation period the subjects participated in a strength training and cardiovascular training program four days per week (62). Strength exercises were performed at 10 exercises per session, with 8-12 sets per exercise and 2-10 repetitions per set (62). The authors reported that HMB supplementation did not alter muscular strength, total body weight, or percent body fat in collegiate football players undergoing a exercise program (62). It was concluded that HMB may be most effective when initiating an exercise training program or increasing the workload of a training program (62).

The second HMB research study that involved trained athletes was conducted by O'Connor and Crowe and elite male rugby players were used as subjects (50). In this study aerobic power was tested using a 20 meter shuttle test and anaerobic capacity was measured using a 60 second maximal cycle ergometer test (50). Pre and post-supplement measurements were separated by six weeks and the dosage was 3g per day (50). It was reported that HMB supplementation did not alter aerobic or anaerobic power in the subjects (50).

O’Connor and Crowe conducted another study using elite male rugby players and HMB supplementation and used the same dosage and supplementation time period (48). In this study muscular strength and endurance, leg power, and anthropometry were measured pre and post-supplement (48). The authors reported no improvement in muscular strength and endurance, leg power, or anthropometry with HMB supplementation during a resistance training program (48). The authors concluded that the benefits from HMB may be limited in well trained subjects, particularly those with a resistance training background (48).

A study conducted by Kreider et al. examined the effects of HMB supplementation during resistance training on markers of catabolism, body composition,
and strength in experienced resistance trained males (29). The supplementation period was 28 days and the dosage of HMB was 0, 3, or 6g per day for this study (29). Pre and post supplementation measurements were taken and consisted of body composition, venous and urine samples, and bench press and leg press maximal lifts (29). The authors reported that no dosage of HMB affected markers of anabolic/catabolic status, body composition, or gains in upper and lower body strength (29).

Slater et al. conducted a study with resistance trained males and 6 weeks supplementation of 3g per day of HMB (69). Pre and post supplementation measurements were taken and consisted of body composition, venous blood sample, and 3 repetition maximum strength tests in the bench press, leg press, and chin-ups (69). It was reported that HMB did not influence changes in strength or body composition during resistance training in strength trained subjects (69).

A second group of studies were found that reported HMB supplementation did not have any benefits and these studies all had supplementation periods less than 14 days (Table 2.1). The first of these studies was conducted by Paddon-Jones et al. and the HMB supplementation was 40mg/kg bodyweight/day for 6 days (52). The exercise protocol for this study was 24 maximal voluntary eccentric contractions of the elbow flexors and pre and post-test measurements were taken (52). The measurements used in this study were muscle soreness, upper arm girth, and isometric, concentric, and eccentric torque (52). The authors reported that short-term supplementation with HMB did not reduce the severity of muscle soreness or improve the recovery of muscle torque following an acute bout of eccentric exercise (52).

Another short-term HMB supplementation study was conducted by Hoffman et al. (20). In this study Division III college football players supplemented with 3g per day HMB for 10 days during their preseason football training camp (20). Pre and post-supplementation measures were taken and included blood samples measured for testosterone, cortisol, myoglobin, and creatine kinase, and a 30 second Wingate anaerobic power test was used to measure peak power, mean power, and rate of fatigue (20). The authors reported that short-term HMB supplementation did not attenuate biochemical markers of muscle damage or improve anaerobic power in college football players (20). The authors concluded that if HMB has any ergogenic benefit in attenuating muscle damage, it is likely to be most effective in the untrained population where the potential for muscle damage to occur during exercise is greatest (20).
A third short-term HMB supplementation study was conducted by Nunan et al. and subjects supplemented for 14 days with 3g per day HMB (46). Post-test measures were taken 24, 48, and 72 hours after a 40-minute downhill run and consisted of mid-thigh girth, knee extensor range of motion, serum CK activity, isometric and concentric torque, and a DOMS measure (46). The authors reported that HMB supplementation had no effect on any of the indices of muscle damage but, there was a trend for a more rapid recovery of isometric function (46).
Table 2.1: Summary of Studies that Reported no Effects of HMB Supplementation

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subjects</th>
<th>Exercise Protocol</th>
<th>Supplementation Duration</th>
<th>Dosage</th>
<th>Outcome Measurements</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ransone et al. (62)</td>
<td>35 College football players</td>
<td>Strength training: 8-12 sets, 2-10 repetitions for each exercise</td>
<td>28 Days</td>
<td>3g Per day</td>
<td>1) 3-5 Repetition maximum lifts: Bench press, power cleans, squats 2) Body composition: 3 site skinfold</td>
<td>1) HMB did not alter muscular strength, total body weight, or percent body fat</td>
</tr>
<tr>
<td>O’Connor and Crowe (50)</td>
<td>27 Professional rugby players</td>
<td>Pre and post measurements only</td>
<td>6 Weeks</td>
<td>3g Per day</td>
<td>1) 20 meter shuttle aerobic power test 2) 60 second cycle anaerobic capacity test with blood lactate</td>
<td>1) HMB did not alter aerobic or anaerobic power</td>
</tr>
<tr>
<td>O’Connor and Crowe (48)</td>
<td>30 Professional rugby players</td>
<td>Pre and post measurements only – Subjects continued normal workout program</td>
<td>6 Weeks</td>
<td>3g Per day</td>
<td>1) 3 Repetition maximum: bench press, deadlift, shoulder press, prone row 2) Leg power: 10s maximal cycle ergometer test 3) Anthropometric profile</td>
<td>1) HMB did not alter muscular strength and endurance, leg power, or anthropometry</td>
</tr>
<tr>
<td>Kreider et al. (29)</td>
<td>40 Resistance trained males</td>
<td>Pre and post measurements only – Subjects continued normal workout program</td>
<td>28 Days</td>
<td>0, 3, or 6g Per day</td>
<td>1) Venous and urine sample 2) Maximum strength tests: Bench press, leg press 3) Body composition</td>
<td>1) HMB did not affect markers of anabolic/catabolic status, body composition, or upper and lower body strength</td>
</tr>
<tr>
<td>Slater et al. (69)</td>
<td>27 Resistance trained males</td>
<td>Pre and post measurements only – Subjects continued normal workout program</td>
<td>6 Weeks</td>
<td>3g Per day</td>
<td>1) Venous blood sample 2) Body composition 3) 3 repetition maximum strength tests: Bench press, leg press, chin-ups</td>
<td>1) HMB had no influence on strength gains or body composition</td>
</tr>
<tr>
<td>Paddon-Jones et al. (52)</td>
<td>17 Non-resistance trained males</td>
<td>24 Maximal voluntary eccentric contractions of the elbow flexors</td>
<td>6 Days</td>
<td>40mg/kg bodyweight/day</td>
<td>1) Muscle soreness 2) Upper arm girth 3) Isometric, concentric, and eccentric torque</td>
<td>1) Short-term HMB did not reduce muscle soreness or improve recovery of muscle torque following an acute bout of eccentric exercise</td>
</tr>
<tr>
<td>Hoffman et al. (20)</td>
<td>26 Division III college football players</td>
<td>Preseason football training camp</td>
<td>10 Days</td>
<td>3g per day</td>
<td>1) Blood measures: Testosterone, cortisol, myoglobin, creatine kinase 2) Anaerobic power performance: 30 sec Wingate test</td>
<td>1) HMB did not attenuate markers of muscle damage or improve anaerobic power performance</td>
</tr>
<tr>
<td>Nunan et al. (46)</td>
<td>14 Male recreational exercisers</td>
<td>40-minute downhill run</td>
<td>14 Days</td>
<td>3g per day</td>
<td>1) Mid-thigh girth 2) Knee range of motion 3) Serum CK activity 4) Isometric and concentric torque 5) DOMS measurement</td>
<td>1) HMB had no effect on indices of muscle damage 2) There was a trend for a more rapid recovery of isometric function with HMB</td>
</tr>
</tbody>
</table>
Research Studies That Reported Positive Effects of HMB Supplementation

HMB supplementation has been claimed to enhance gains in strength and lean body mass associated with resistance training and it has been suggested to accomplish this through a reduction in skeletal muscle damage (73). Because of this the research studies that have reported positive effects of HMB are grouped into two categories, ones that show a decrease in muscle damage and ones that show an increase in muscular strength or lean body mass (Table 2.2).

The first study to report a decrease in muscle damage caused by HMB supplementation was conducted by Ostaszewski et al. and rat and chicken whole muscles were used in vitro (51). This study did not utilize an exercise protocol rather; protein synthesis and breakdown were measured in whole muscles (51). The authors reported that HMB decreased proteolysis in both rat and chicken muscles but did not affect protein synthesis in either group (51).

A human resistance training study with HMB supplementation was conducted by Panton et al. (56). In this study both males and females were used and they were grouped into a trained or untrained groups (56). In the male group creatine phosphokinase (CK), which is a marker for muscle damage, increased only 3.7% from baseline in the HMB group but, showed a 15.2% increase in the placebo group during the exercise protocol (56). For females, the difference in plasma CK was greater between the HMB supplemented and placebo groups, with the HMB group decreasing by 12.8% and the placebo increasing by 46.9% from baseline (56). In the male cohort the HMB group had a greater increase in upper body strength compared to the placebo (placebo 7.0 kg and HMB 9.9 kg; p ≤ 0.05) and the female HMB group showed a trend toward a greater increase in upper body strength compared to the placebo (placebo 3.2 kg and HMB 5.1 kg; p=0.065) (56). The authors reported that the response from HMB does not appear to be dependent on previous training levels and both males and females appear to respond to HMB supplementation (56). The authors concluded that both males and females in the HMB group, tended to have lower CK levels than the placebo group indicating that less cell membrane damage may have occurred (56). It has been postulated that supplementation with HMB can provide a source of HMG-CoA for cholesterol synthesis and this cholesterol is an important constituent of cell membranes (56). Therefore, supplementation with HMB may lead to increased
membrane integrity by supplying a source for increased synthesis of intracellular cholesterol during a time of need (56).

Van Someren et al. conducted a study using non-resistance trained males and supplemented with both HMB and KIC for 14 days (79). The subjects performed an exercise protocol designed to induce muscle damage using single arm biceps curls and outcome measurements were taken at: pre-exercise, and 1h, 24h, 48h, and 72h post-exercise (79). It was reported that there was a significant difference between HMB/KIC and placebo groups for the percentage change in 1 repetition maximum in the biceps curl (P ≤ 0.05) (79). Also, there was a significant difference in the CK response between the groups, which increased over the 48h following exercise in the placebo group but, in the HMB/KIC group CK levels showed very little increase after exercise (79). The percentage increase in limb girth was attenuated in the HMB/KIC group over the 72h post-exercise period and it returned to pre-exercise levels faster than the placebo group (79). Finally, DOMS was significantly lower at 24h post-exercise in the HMB/KIC group when compared to the placebo group (79). The authors concluded that HMB and KIC supplementation attenuated muscle damage in non-resistance trained males following a single bout of eccentric resistance exercise (79). Also, HMB/KIC had a significant effect on the percentage decrement in muscle function demonstrated by a greater decrement in 1 repetition maximum strength in the placebo group over the recovery period (79). The attenuated CK response in the HMB/KIC group indicated that muscle damage was reduced following the exercise protocol, perhaps via enhanced cell membrane integrity (79).

Knitter et al. examined the effects of HMB supplementation on muscle damage after a prolonged run (27). After 6 weeks of supplementation subjects completed a 20km run and plasma creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) activity were measured as markers of muscle damage (27). The HMB group experienced a significantly lower CPK response (P = 0.05) and lower LDH levels (P = 0.003) compared with the placebo group over the 4 days after the prolonged run (27). The authors concluded that because both groups performed equally well in the prolonged run, the larger increases in enzyme activity in the placebo group suggest that they sustained more muscle damage as a result of the run (27). These findings are consistent with the hypothesis that HMB supplemented subjects experienced less
muscle damage or that they recovered from muscle damage at a faster rate when compared to a placebo group (27).

Another study that examined HMB supplementation during a resistance training program was conducted by Nissen et al. (39). In this study three groups of subjects weight lifted for 3 weeks and supplemented with either 0, 1.5, or 3g per day of HMB (39). The authors reported that the increase in lower body weight lifted during the study was greater in the HMB groups than in the unsupplemented group (P < 0.01) (39). The unsupplemented subjects increased the number of abdominal efforts by 14% during the 3 weeks, whereas both HMB groups increased the number of abdominal efforts approximately 50% over the 3 week study (P < 0.05) (39). Also, after one week of exercise urinary methylhistidine (MH), a marker for muscle damage, increased 94% in subjects not supplemented with HMB, 85% in subjects supplemented with 1.5g HMB, and 50% in subjects supplemented with 3g HMB (39). In week two MH was 27% above basal level in the control group, and 4 and 15% below the basal level for the 1.5 and 3g HMB groups (39). During the third week of the study, HMB did not have a significant effect on MH (39). The authors concluded that these changes suggest that HMB prevents or slows muscle damage as well as partially preventing the increase in proteolysis associated with intense muscular work (39).

The second group of research studies that have reported positive effects of HMB supplementation are ones that have reported increases in strength and/or increases in fat free mass (Table 2.2). The first of these studies was conducted by Pinheiro et al. and evaluated the effects of HMB supplementation for 4 weeks upon metabolic parameters and skeletal muscle contractile function in rats (57). It was reported that HMB supplementation increased muscle tetanic force by 18.2% (P = 0.042) and increased the resistance to acute fatigue in the gastrocnemius muscle (P < 0.05) (57). Since fatigue has been associated with increased muscle damage induced by contractile activity, the authors suggested that HMB supplementation decreased exercise induced muscle damage, due to its increased resistance to acute fatigue (57). This protective effect of HMB against contractile activity induced damage may be associated to increased stability of muscle plasma membrane (57). The authors concluded that HMB supplementation is associated with increased intrinsic force capacity of single skeletal muscle fibers and prevention of acute muscle fatigue (57).
Gallagher et al. employed an 8 week supplementation period with either 0, 3, or 6g of HMB per day during a resistance training program to determine whether a higher dose of HMB provided any additional benefits (16). It was reported that HMB supplementation elicited no differences in one repetition maximum strength compared with the placebo group (17). But, the 3g per day HMB group improved isometric knee extension torque by 32% which represented a greater improvement than the 0 and 6g per day groups (P < 0.05) (16). The CK values taken at 48h after the initial exercise bout were increased in the 0g per day group when compared to the other two groups (P < 0.05) (16). Also, the 3g per day group demonstrated a greater increase in fat free mass (1.9kg) from pre to post-training than the other two groups (P < 0.05) (16). The authors concluded that HMB supplementation appears to increase peak isometric torque, and fat free mass, and decrease plasma CK activity (16). Also, higher doses of HMB (6g per day) do not promote greater strength or fat free mass gains (16).

Portal et al. assessed the effect of 7 weeks of HMB supplementation on elite male and female junior volleyball players during the initial phase of the training season (59). It was reported that HMB supplementation resulted in a significantly greater increase of fat-free mass (P = 0.00), significantly greater decrease in percent body fat (P = 0.04) and a significantly greater strength gain in 6 repetition maximum bench press (P = 0.00) and leg press (P = 0.00) (59). HMB supplementation also resulted in a significant greater increase in peak (P = 0.00) and mean (P = 0.00) anaerobic power (59). The authors concluded that HMB supplementation was associated with a greater increase in muscle mass, muscle strength, and anaerobic properties in elite male and female adolescent volleyball players (59).

A study conducted by Thomson et al. determined the effects of nine weeks of HMB supplementation during a resistance training program on the strength and body composition of resistance trained males (76). It was reported that HMB supplementation substantially increased leg extension strength (9.1 ± 7.5 kg, P = 0.05) but, the effect on bench press (-1.9 ± 9.3kg, P = 0.72) and bicep preacher curl (-1.7 ± 4.7 kg, P = 0.51) was unclear (76). Also, the overall combined lift strength was trivial (1.6 ± 4.3 kg, P = 0.52) (76). The increase in body mass (P = 0.35) and fat free mass (P = 0.84) were negligible (76). The authors suggested that if HMB acts via accentuating cholesterol synthesis and is used as a structural component of cell membranes, it may support membrane function that is essential for excitation and optimal contractile performance.
during resistance exercise (76). The authors concluded that HMB supplementation in conjunction with resistance training may result in meaningful improvements in lower body strength and benefits to lean mass are unlikely, but small reductions in fat mass are possible in resistance trained males (76).
<table>
<thead>
<tr>
<th>Authors</th>
<th>Subjects</th>
<th>Exercise Protocol</th>
<th>Supplementation Duration</th>
<th>HMB Dosage</th>
<th>Outcome Measurements</th>
<th>Findings</th>
</tr>
</thead>
</table>
| Ostaszewski et al. (51)     | Rat and chicken whole muscles         | None – Protein synthesis and breakdown were measured   | Muscles incubated in HMB solution for 2 hours | Muscles incubated in 1mM HMB solution   | 1) Protein synthesis  
2) Protein breakdown                                     | 1) HMB inhibited proteolysis                                             |
| Panton et al. (56)          | 43 Males and 41 females – Trained and untrained groups | Resistance training – 4 weeks, 3 times per week, 3 sets of 3-6 repetitions | 4 Weeks | 3g per day | 1) Plasma creatine phosphokinase  
2) Body composition  
3) Strength tests – 1 Repetition maximum – Bench press, leg press, leg extension | 1) Both males and females respond to HMB  
2) HMB response did not appear to be dependent on previous training levels |
| Van Someren et al. (79)     | 6 Non-resistance trained males         | 3 sets of 10 repetitions of single arm eccentric biceps curls at 70% 1 repetition maximum | 14 Days | HMB - 3g per day KIC – 0.3g per day | 1) Concentric 1 repetition maximum  
2) Plasma creatine kinase activity  
3) Muscle soreness (DOMS)  
4) Limb girth  
5) Range of Motion | 1) HMB and KIC attenuated muscle damage  
2) HMB/KIC attenuated increase in creatine kinase, DOMS, and limb girth |
| Knitter et al. (27)         | 8 Males and 8 Females                 | 20km prolonged run                                     | 6 Weeks | 3g per day | 1) Maximal oxygen consumption (Vo\textsubscript{2} max)  
2) Body composition  
3) Plasma creatine phosphokinase  
4) Lactate dehydrogenase | 1) HMB group showed lower levels of creatine phosphokinase and lactate dehydrogenase when compared to a placebo group |
| Nissen et al. (39)          | 41 Males                              | Resistance training – 3 days per week, 3 sets of 3-5 repetitions | 3 Weeks | 1.5g or 3.0g per day | 1) Methylhistidine activity  
2) Lactate dehydrogenase  
3) Body Composition  
4) Fat free mass | 1) HMB groups increased abdominal exercises and lower body weight lifted to a greater degree  
2) HMB groups had smaller methylhistidine activity increases |
| Pinheiro et al. (57)        | 24 Male Wistar rats                   | None – Pre and post supplementation measures only      | 4 Weeks | 320 mg/kg per day | 1) Muscle twitch force  
2) Tetcnic muscle force | 1) HMB increased tetanic forces by 18.2%  
2) HMB increased resistance to acute fatigue in the gastrocnemius muscle |
| Gallagher et al. (16)       | 37 Untrained males                    | Resistance training – 3 sets of 10 repetitions and a final set to failure | 8 Weeks | 0, 3, or 6g per day | 1) Maximal isometric contractions  
2) Concentric and eccentric isokinetic evaluation  
3) Fatigue evaluation  
4) Plasma creatine phosphokinase  
5) Body composition | 1) HMB elicited no differences in one repetition maximum  
2) 3g per day HMB group improved isometric knee extension torque  
3) 3g per day HMB group had a greater increase in fat free mass |
| Portal et al. (59)          | 15 male and 14 female elite junior volleyball players (13.5 – 18 years old) | Resistance training – 65-75% of one repetition maximum  
Endurance training – long distance running | 7 Weeks | 3g per day | 1) Body composition  
2) Muscle strength – Upper and lower body 6 repetition maximum tests  
3) Anaerobic power – 30s Wingate test  
4) Aerobic power – 20 min shuttle run test  
5) Hormones and inflammatory mediators | 1) HMB was associated with a greater increase in muscle mass, muscle strength, and anaerobic properties |
| Thomson et al. (76)         | 22 Resistance trained males           | Resistance training – 3 sessions per week, 2-3 sets, 5-15 repetitions | 9 Weeks | 3g per day | 1) Strength – 1 repetition maximum – Bicep curl, bench press, leg extension  
2) Body composition | 1) HMB increased leg extension strength  
2) Overall combined lift strength was trivial  
3) Increase in body mass and fat-free mass was negligible |
Even though there cannot be any clear cut conclusions drawn from the current research on HMB supplementation, there can be some general guidelines drawn. Most of the studies used a dosage of 3g per day for HMB supplementation and it appears that short term use and increased dosing are not useful (54, 73). Inconsistent gains in upper vs. lower body strength have been observed with the majority of the improvements being in lower body strength (66). Supplementation gains may also be related to the training status of the subjects involved, with greater gains more likely seen in less trained subjects (54). There is a pattern for reduction in markers of muscle damage with HMB supplementation but, the magnitudes are variable (66). It is believed that HMB supplementation exerts positive effects in conditions in which muscle proteolysis is more pronounced, such as sedentary individuals and persons with AIDS or cancer (54, 91). Also, the type of muscle contraction may play a role in the amount of gains that are seen with HMB, with eccentric contractions seeing more gains, due to higher muscle tension and greater muscle damage (91). There have been no adverse effects on hematology, or hepatic or renal function with HMB supplementation and some studies reported a net decrease in total cholesterol, low density lipoprotein cholesterol, and systolic blood pressure (18, 43). Also, at this time HMB is currently not banned from any federal, international, or other sporting organizations (54).

**Conclusion**

Peripheral physiological muscle fatigue is a problem that affects many other people besides athletes and those partaking in exercise programs. Muscle fatigue can affect those suffering from long term illnesses such as AIDS and cancer or even young, middle, and older aged adults completing their activities of daily living. The mechanisms of muscle fatigue are not know for sure but, most current research points to a defect in E-C coupling as the main source of muscle fatigue. This E-C uncoupling is the main cause of reduced muscle force and LFF which can last for several days. This LFF is most often caused by eccentric muscle contractions which cause more muscle damage than concentric or isometric contractions. Eccentric contractions affect everyone everyday of their lives. Just walking on a level surface involves a great deal of eccentric muscle contractions. If someone engages in an increase of eccentric contractions such as increased walking, walking downhill, or a more structured exercise program it is very likely they will experience increased muscle damage and increased LFF. If a nutritional supplement can be used to reduce this muscle damage, caused by eccentric muscle
contractions, then the decreased force production of LFF can be avoided. HMB supplementation has been suggested and shown to reduce muscle damage during activity. If supplementation with this substance can be shown to reduce LFF caused by eccentric muscle contractions then it may provide quicker recovery from physical activity for recreational athletes, the general public, and people suffering from long term illnesses.

Some studies demonstrate that HMB has the ability to increase strength gains and fat free mass gains during a resistance training program. It may accomplish this through reducing the amount of muscle fatigue after one bout of physical activity, allowing the muscle to perform more work in a repeated bout of activity, therefore producing a greater training effect and greater gains in strength and fat free mass. But, to date there have not been any studies determining HMB’s ability to reduce muscle fatigue after a bout of physical activity. Also, there does seem to be reduced markers of muscle damage with HMB supplementation (17, 40, 55, 80). So, HMB supplementation may be effective in reducing LFF caused by muscle damage. To this date a research study has not been found investigating the effects of HMB supplementation and the reduction of LFF. Studies have shown that there are changes to rapid muscle force characteristics after a bout of physical activity. But, there have not been any studies that have tried to reduce these changes through nutritional supplements or any other means. So, future research should be focused on HMB’s ability to reduce changes in rapid muscle force characteristics and long term muscle fatigue.
Chapter III: Methodology

Subjects

A total of 33 healthy recreationally active (physically active for 60 mins per day 3-5 days per week) young (18-35 yrs old) adults (15 women and 18 men) participated in this study. After providing written informed consent approved by the University of Kentucky Office of Research Integrity Medical Institutional Review Board, each subject filled out a Physical Activity Readiness-Questionnaire and Health History Form to identify any existing contraindications to the testing procedures as specified in the American College of Sports Medicine Guidelines for Exercise Testing and Prescription (1). Additional contraindications to inclusion in this study were previous lower limb orthopedic injuries and/or limitations prohibiting completion of the muscle testing or fatiguing protocols included in this investigation.

Testing Procedures

Prior to all pre and post supplementing muscle testing sessions, all subjects completed a familiarization session. This was followed by scheduling and completion of the anthropometric and body composition measurement testing, Pre-Supplement baseline session with fatigue protocol, Pre-Supplement 48 hour recovery session, and Pre-Supplement 96 hour recovery session. The Pre-Supplement measures were performed on a randomly selected lower limb of each subject. Identical testing procedures were then performed on the opposing lower limb following 3 wks of supplementation. This supplementation consisted of a 1 gram tablets consumed 3 times daily (morning, mid-day, and evening) of calcium β-Hydroxy-β-Methylbutyrate (HMB), for a total of 3 grams per day. The Post-Supplement testing provided Post-Supplement baseline session with fatigue protocol, Post-Supplement 48 hour recovery session, and Post-Supplement 96 hour recovery session, thus allowing a repeated measures between lower limb muscle testing study design. During each testing period maximal voluntary isometric contraction (MVC), peak twitch torque, 10/50 Hz ratio stimulation, and EMG measurements were taken. After the baseline with fatigue protocol session the subject returned on two more occasions at approximately 48 and 96 hours after the fatigue protocol to complete the recovery measurements. While participating in this study, subjects were asked to maintain their current exercise and dietary patterns and to abstain from other nutritional supplements and energy drink consumption. Twenty-four hours prior to Pre and Post Supplement baseline sessions with fatigue protocol, subjects
were asked to refrain from nonprescription drugs, exercise, alcohol, energy drink consumption, and caffeine.

**Anthropometric and Body Composition Measurements.** Standing height and body weight were determined for each subject using a wall-fixed stadiometer (Seca Statiometer Pat. No. 4694581) and a calibrated scale (Teraoka D1-10; Singapore), respectively. A total body dual energy x-ray absorptiometry (DXA) scan was performed on each subject using a Lunar Prodigy (Lunar Inc., Madison, WI) densitometer to determine relative adiposity (%Fat). All scans were analyzed by a single trained investigator using Lunar software version 14.10. In addition, range of motion measurements of the ankle were taken using a transparent plastic goniometer (Baseline, Diagnostic and Measuring Instruments, White Plains, NY) immediately prior to the muscle testing performed on Pre- and Post-Supplement baseline sessions, 48 hour recovery sessions, and 96 hour recovery sessions.

**Pre and Post Supplement Baseline Session with Fatigue Protocol**

Each baseline session with fatigue protocol was preceded by a 5 min low-intensity warm-up performed on a recumbent cycle ergometer. This was followed by baseline isometric skeletal muscle strength (MVC) and skeletal muscle activation measures (EMG). Electromyography was used simultaneously throughout the skeletal muscle measures. Following the Pre and Post Supplement baseline measures, a muscle fatigue protocol was implemented followed by identical isometric skeletal muscle strength, skeletal muscle activation and EMG measures. This process of imposing a muscle fatiguing protocol followed by skeletal muscle strength, activation and EMG measures was repeated a total of 4 times (4 sets of 25 repetitions) with 15 seconds rest between fatiguing and measurement sets. To complete Pre and Post Supplement baseline session with fatigue protocol, muscle strength, muscle activation and EMG measures were again taken at 2, 5, 10, 15, and 20 mins following the final fatiguing bout to determine the acute recovery measures.
Isometric Skeletal Muscle Strength

Isometric strength of the tibialis anterior was obtained from the Biodex Multi-Joint System 4 isokinetic dynamometer (Biodex Medical Systems, Shirley, NY) with the Biodex Advantage software version 4.0. All isometric tests were administered by a single trained investigator. Participants sat in a slightly reclined position with the hip, knee, and ankle angles set at 100, 140, and 30 degrees plantar flexion. The participants were stabilized with 2 shoulder straps, a waist strap, and a thigh strap. All voluntary and evoked isometric contractions were performed at an ankle joint angle of 30 degrees of plantar flexion. Before testing, familiarization with the equipment and testing procedures were completed by having the participant perform three submaximal contractions on each strength test. For the test procedures, each participant was asked to complete three nonconsecutive maximal isometric contractions (ankle joint angle of 30 degrees...
plantar flexion). There were 120 seconds rest between each maximal isometric contraction. Each participant was instructed to exert maximal voluntary effort by contracting as hard and as fast as possible during the isometric contractions. The highest contraction amplitude value of the three attempts for each exercise was recorded and used in the subsequent analyses.

**Electromyography**

To monitor changes in muscle electrical activity during isometric strength testing and the development of fatigue, electromyographic signals were collected. Prior to electrode placement, the skin was shaved of any hair and cleaned with pre-soaked alcohol swabs. Surface electrodes (Delsys system Bagnoli DE-2.1 single differential electrodes, inter-electrode distance 10 mm, bandwidth 20-450 Hz, Natick, MA) were placed on the tibialis anterior (~ 7 cm distal to the tibial tuberosity and ~ 2 cm lateral to the tibial anterior border) and soleus muscles (~ 2 cm distal to the medial head of the gastrocnemius). Data was collected using the Delsys Bagnoli 8-channel digital interface (Model number: DS-B03, Natick, MA). The gain setting was 1000 Hz and the sampling rate was 1000 Hz. The ground electrode was place on the patella of the limb that was not being tested. At the peak of each MVC the root mean squared value was recorded for one second. In addition, all raw EMG data was smoothed with a fourth order Butterworth filter.

**Skeletal Muscle Activation**

Stimulated contractions of the dorsiflexors were evoked electrically with two round carbon rubber electrodes (3 cm diameter; Medi-Stim, Wabasha, Minnesota, USA), positioned at the optimal site for twitch torque. The anode was positioned anterior, and the cathode 2-3 cm inferior to the tibial tuberosity over the deep branch of the common fibular nerve. A computer-triggered stimulator (model DS7AH, Digitimer, WelwynGarden City, Hertfordshire, UK) set at 400V provided the electrical stimulation using a pulse width of 100 ms.

Peak twitch torque was determined by increasing the current until a plateau in dorsiflexor peak torque was reached and then the current was further increased by at least 15% to ensure supramaximal stimulation was achieved. This stimulation intensity was used to evoke a twitch (on pulse) and a doublet (two pulses at a 10 ms interpulse interval) to assess potentiation and voluntary activation, respectively. Peak 50Hz torque
was determined by increasing the current until a plateau in dorsiflexor peak torque was achieved. Prolonged low-frequency force depression was accessed via the 10Hz (5 pulses over 0.5s) and 50Hz stimulus (25 pulses over 0.5s) peak torque ratio.

Fatigue Protocol

All voluntary and evoked isometric dorsiflexion contractions were performed at an ankle joint angle of 30° plantarflexion (PF) (21). Eccentric or lengthening contractions began at the neutral ankle angle (0° PF) and continued until 30 degrees of plantarflexion. The fatigue protocol consisted of 4 sets of 25 eccentric isokinetic dorsiflexion contractions at 30°/s through a 30 degree range of motion with each set separated by ~ 30 s of rest (21). Dominate or non-dominate lower limb was randomized. During this fatigue protocol EMG measurements were recorded. Immediately after the last set, the post fatigue measurements were taken and consist of: MVC peak torque, peak twitch torque, and 10/50 Hz ratio stimulation. These measurements were taken again during the recovery period at: 2, 5, 10, 15, and 20 minutes after the fatigue protocol.

Supplementation

ON™ HMB is a commercially available nutritional supplement for adult use only. Participants consumed one tablet three times per day (morning, mid-day, and evening) containing a total of 3000 mg of calcium β-Hydroxy-β-Methylbutyrate.

Statistical Analysis

Mean differences between groups were examined by using a repeated measures ANOVA and the level of significance was set at P < 0.05. When significant differences were observed a post hoc analysis using a Bonferroni correction was used to determine where significant differences existed. All data analyses were completed using SAS 9.3.
Chapter IV: Results

A total of 33 subjects enrolled in this study (15 Females, 18 males). Of the 15 female subjects enrolled, 12 completed all testing procedures and 3 failed to complete the post-supplement measures due to lack of compliance. Of the 18 male subjects enrolled, 14 completed all testing procedures and 4 failed to complete the final post-supplement (96-hours recovery) visit due to temporary testing equipment mechanical failure or illness. Subject characteristics are shown in Table 4.1.

Table 4.1: Subject Characteristic Measurements

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>23.2 ± 4.3</td>
<td>18 – 34</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.1 ± 18.3</td>
<td>155.0 – 188.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.1 ± 15.8</td>
<td>52.9 – 126.5</td>
</tr>
<tr>
<td>BMI</td>
<td>25.2 ± 4.1</td>
<td>20.0 – 36.4</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>22.5 ± 9.5</td>
<td>5.6 – 39.8</td>
</tr>
</tbody>
</table>

There were significant measurement differences for the MVC torque, 10 Hz Peak Torque, 50 Hz Peak Torque, 10/50 Hz Torque Ratio, and TA EMG variable measures. The results of these measurement differences are summarized below. However, there were no other variables that showed significant differences between pre-supplement and post-supplement measures, or over any of the measurement times.

10/50 Hz Torque Ratio

There was a significant difference in the 10/50 Hz torque ratio over time (p<0.0001) and between the pre-supplement limb and the post-supplement limb (p=0.005; Table 4.2). There was no significant difference between limb 10/50 Hz torque ratios during the fatigue protocol or the 20-minute recovery period (p=0.16) but, there was a significant difference at the 48-hour and 96-hour recovery measurement times (p=0.005).

The baseline 10/50 Hz torque ratio for the pre-supplement limb was 0.51 ± 0.14 and for the post-supplement limb it was 0.49 ± 0.11 (Table 4.2). At the end of the fourth set of the fatigue protocol it was reduced to 63% of baseline for the pre-supplement limb.
Therefore, after the fatigue protocol, both the pre-supplement limb and the post-supplement limb had a significant relative 10/50 Hz torque ratio deficit. During the recovery period the 10/50 Hz torque ratio continued to decrease at a greater rate in both limbs. At the 20-minute recovery measurement time it was reduced to 49% of baseline in the pre-supplement limb (p<0.0001) and 57% of baseline in the post-supplement limb (p<0.0001; Table 4.2). Therefore, after 20 minutes of recovery both the pre-supplement limb and the post-supplement limb had a significant relative 10/50 Hz torque ratio deficit.

At the 48-hour recovery measurement time, the 10/50 Hz torque ratio in the pre-supplement limb was 0.40 ± 0.12 and for the post-supplement limb it was 0.47 ± 0.11 (Table 4.2). At this measurement time the 10/50 Hz torque ratio, in the pre-supplement limb, was reduced to 78% of baseline (p<0.0001) and the post-supplement limb was reduced to 96% of baseline (p=0.38; Table 4.2). Therefore, after 48 hours of recovery, the pre-supplement limb had a significant relative 10/50 Hz torque ratio deficit and the post-supplement limb had no significant relative 10/50 Hz torque ratio deficits.

The 10/50 Hz torque ratio in the pre-supplement limb at the 96-hour recovery measurement time was 0.44 ± 0.14 and for the post-supplement limb it was 0.46 ± 0.12 (Table 4.2). At this measurement time the 10/50 Hz torque ratio, in the pre-supplement limb, was reduced to 86% of baseline (p=0.02) and in the post-supplement limb it was 94% of baseline (p=0.31; Table 4.2). Therefore, after 96 hours of recovery, the pre-supplement limb had a significant relative 10/50 Hz torque ratio deficit and the post-supplement limb had no significant relative 10/50 Hz torque ratio deficits.

10 Hz Peak Torque

There was a significant difference in 10 Hz peak torque over time (p<0.0001) and between the pre-supplement limb and the post-supplement limb (p=0.0008; Table 4.2). There was no significant difference between limbs during the fatigue protocol or the 20-minute recovery period (p=0.89; Table 4.2). However, there was a significant difference at the 48-hour and 96-hour recovery measurement times (p=0.0008; Table 4.2).

The baseline 10 Hz peak torque for the pre-supplement limb was 4.8 ± 2.2 Nm and for the post-supplement limb it was 4.7 ± 2.4 Nm (Table 4.2). At the end of the fourth set of the fatigue protocol the 10 Hz torque was reduced to 56% of baseline in the pre-supplement limb (p<0.0001) and in the post-supplement limb it was reduced to 56%
of baseline (p<0.0001; Table 4.2). Therefore, after the fatigue protocol, both the pre-supplement limb and the post-supplement limb had a significant relative 10 Hz peak torque deficit. The reduction in the 10 Hz peak torque continued throughout the recovery period, and at the 20-minute recovery measurement time it was 47% of baseline for the pre-supplement limb (p<0.0001) and 44% of baseline for the post-supplement limb (p<0.0001; Table 4.2). Therefore, after 20-minutes of recovery, both the pre-supplement limb and the post-supplement limb still had a significant relative 10 Hz peak torque deficit.

The 10 Hz peak torque in the pre-supplement limb at the 48-hour recovery measurement time was 3.7 ± 2.1 Nm and for the post-supplement limb it was 4.4 ± 1.9 Nm (Table 4.2). At the 48-hour recovery measurement time, in the pre-supplement limb, the 10 Hz torque was reduced to 77% of baseline (p<0.0001) and the post-supplement limb was reduced to 94% of baseline (p=0.26; Table 4.2). Therefore, after 48 hours of recovery, the pre-supplement limb had a significant relative 10 Hz peak torque deficit and the post-supplement limb had no significant relative 10 Hz peak torque deficits.

The 10 Hz peak torque in the pre-supplement limb at the 96-hour recovery measurement time was 4.2 ± 2.4 Nm and for the post-supplement limb it was 4.3 ± 2.2 Nm (Table 4.2). At the 96-hour recovery measurement time the 10 Hz torque in the pre-supplement limb was reduced to 88% of baseline (p=0.05), and for the post-supplement limb it was 94% of baseline (p=0.53; Table 4.2). In addition, there was a significant increase in 10 Hz peak torque from the 48-hour recovery measurement time to the 96-hour recovery measurement time in the pre-supplement limb (p=0.02; Table 4.2). Therefore, after 96 hours of recovery, the pre-supplement limb had a significant relative 10 Hz peak torque deficit and the post-supplement limb had no significant relative 10 Hz peak torque deficits.

**50 Hz Peak Torque**

There was a significant difference in 50 Hz peak torque over time during the fatigue protocol and the 20-minute recovery period (p<0.0001). But, there was no significant difference between baseline and the 48-hour and 96-hour recovery measurement times (p=0.19). Similarly, there was no significant difference in the 50 Hz peak torque between the pre-supplement and post-supplement limbs during the fatigue
protocol and 20-minute recovery period (p=0.80) or between the 48-hour and 96-hour recovery measurement times (p=0.25; Table 4.2).

The baseline 50 Hz peak torque for the pre-supplement limb was 10.1 ± 5.5 Nm and for the post-supplement limb it was 9.8 ± 5.0 Nm (Table 4.2). At the end of the fourth set of the fatigue protocol the 50 Hz peak torque was reduced to 84% of baseline in the pre-supplement limb (p<0.0001) and 80% of baseline in the post-supplement limb (p=0.02; Table 4.2). Therefore, after the fatigue protocol, both the pre-supplement limb and the post-supplement limb had a significant relative 50 Hz peak torque deficit. During the recovery period the 50 Hz peak torque continued to increase and at the 20-minute recovery measurement time it was recovered to 91% of baseline in the pre-supplement limb (p=0.07) and 86% of baseline in the post-supplement limb (p=0.02; Table 4.2). Therefore, after 20 minutes of recovery the pre-supplement limb did not show significant relative 50 Hz peak torque deficits and the post-supplement limb had a significant relative 50 Hz torque deficit.

There were no significant differences in the 50 Hz peak torque at the 48-hour recovery measurement time or the 96-hour recovery measurement time for either the pre-supplement limb (p= 0.18) or the post-supplement limb (p=0.25).

**MVC Torque**

To test the reliability of the MVC torque measurement an interclass correlation coefficient (ICC) and method error was calculated between the familiarization and pre-supplement testing sessions. The ICC for MVC torque was 0.999 and the method error was 0.51 Nm. There was a significant difference in MVC torque over time (p<.0001) and between the pre-supplement limb and the post-supplement limb (p<.0001; Table 4.2). There was no significant difference between limbs during the fatigue protocol or the 20-minute recovery measurement time (p=.48) however, there was a significant difference at the 48-hour and 96-hour recovery measurement times (p<.0001).

At baseline the MVC torque for the pre-supplement limb was significantly higher when compared to the post-supplement limb (p=0.001). The MVC torque for the pre-supplement limb at baseline was 27.6 ± 11.7 Nm and for the post-supplement limb it was 21.9 ± 8.5 Nm (Table 2). After the fourth set of the fatigue protocol in the pre-supplement limb MVC torque was reduced by 13.5 Nm resulting in 51% of the baseline measures (p<.0001). The post-supplement limb was reduced by 10.6 Nm resulting in
52% of baseline measures after the fourth set of the fatigue protocol (p<.0001; Table 4.2). Therefore, after the fatigue protocol, both the pre-supplement and post-supplement limbs had a significant relative MVC torque deficit. After 20-minutes of recovery following the final set of the fatigue protocol, the MVC torque in the pre-supplement limb returned to 17.5 ± 1.5 Nm. At the same measurement time the post-supplement limb returned to 15.7 ± 1.6 Nm. These values were 65% of baseline for the pre-supplement limb and 75% of baseline for the post-supplement limb. The MVC torque for both the pre-supplement limb (p<.0001) and post-supplement limb (p=.003) was still significantly reduced from baseline following the 20-minute recovery time (Table 4.2). Therefore, after 20-minutes of recovery, both the pre-supplement and post-supplement limbs had a significant relative MVC torque deficit.

The MVC torque in the pre-supplement limb at the 48-hour recovery measurement time was 22.3 ± 9.8 Nm resulting in 81% of the baseline measures (p<.0001; Table 4.2). The MVC torque for the post-supplement limb at this same measurement time was 20.5 ± 9.5 Nm resulting in 94% of the baseline measures (p=.53; Table 4.2). Therefore, after 48-hours of recovery, the pre-supplement limb had a significant relative MVC torque deficit and the post-supplement limb had no significant relative MVC torque deficits.

The MVC torque in the pre-supplement limb at the 96-hour recovery measurement time was 22.3 ± 10.2 Nm resulting in 81% of the baseline measures (p<0.0001; Table 4.2). The MVC torque for the post-supplement limb at this same measurement time was 21.3 ± 9.4 Nm resulting in 97% of the baseline measures (p=0.76; Table 4.2). Therefore, after 96 hours of recovery, the pre-supplement limb had a significant relative MVC torque deficit and the post-supplement limb had no significant relative MVC torque deficits.

**TA EMG**

There was a significant difference in TA EMG over time (p<.0001) but, there was no significant difference between the pre-supplement and post-supplement limbs (p=0.08; Table 4.2). Also, at baseline the TA EMG in the pre-supplement limb was significantly higher than the post-supplement limb (p=0.04).

The baseline TA EMG for the pre-supplement limb was 161.3 ± 62.9 µV and for the post-supplement limb it was 141.1 ± 49.5 µV (Table 4.2). At the end of the fourth set
of the fatigue protocol it was reduced to 64% of baseline for the pre-supplement limb (p<0.0001) and 77% of baseline in the post-supplement limb (p<0.0001; Table 4.2). Therefore, after the fatigue protocol, both the pre-supplement limb and the post-supplement limb had a significant relative TA EMG deficit. During the recovery period the TA EMG steadily increased and at the 20-minute recovery measurement time the TA EMG was recovered to 71% of baseline in the pre-supplement limb (p=0.0002) and 81% of baseline in the post-supplement limb (p=0.003; Table 4.2). Therefore, after 20 minutes of recovery, both the pre-supplement limb and the post-supplement limb had a significant relative TA EMG deficit.

The TA EMG in the pre-supplement limb at the 48-hour recovery measurement time was 139.8 ± 64.1 µV and for the post-supplement limb it was 134.5 ± 41.9 µV (Table 4.2). At the 48-hour recovery measurement time the TA EMG was reduced to 86% of baseline (p=0.01) in the pre-supplement limb and 96% of baseline in the post-supplement limb (p=0.064; Table 4.2). Therefore, after 48 hours of recovery, the pre-supplement limb had a significant relative TA EMG deficit and the post-supplement limb had no significant relative TA EMG deficits.

The TA EMG in the pre-supplement limb at the 96-hour recovery measurement time was 139.3 ± 56.1 µV and for the post-supplement limb it was 135.2 ± 47.3 µV (Table 4.2). At the 96-hour recovery measurement time the TA EMG was reduced to 86% of baseline (p=0.01) in the pre-supplement limb and 98% of baseline in the post-supplement limb (p=0.64; Table 4.2). Therefore, after 96 hours of recovery, the pre-supplement limb had a significant relative TA EMG deficit and the post-supplement limb had no significant relative TA EMG deficits.
Table 4.2: Pre-Supplement and Post-Supplement Measures for Fatigue Protocol and Recovery Period

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Eccentric Fatigue Set 1</th>
<th>Eccentric Fatigue Set 2</th>
<th>Eccentric Fatigue Set 3</th>
<th>Eccentric Fatigue Set 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MVC Torque (Nm)</strong></td>
<td></td>
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</tr>
<tr>
<td>Pre-Supplement</td>
<td>27.6 ± 11.7</td>
<td>23.8 ± 12.4</td>
<td>19.1 ± 11.9♦</td>
<td>16.2 ± 9.9**</td>
<td>14.0 ± 9.1†</td>
</tr>
<tr>
<td>Post-Supplement</td>
<td>21.9 ± 8.5</td>
<td>17.4 ± 7.5♦</td>
<td>14.7 ± 8.2♦</td>
<td>13.4 ± 7.3†</td>
<td>11.3 ± 6.8†</td>
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<tr>
<td><strong>10 Hz Peak Torque (Nm)</strong></td>
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</tr>
<tr>
<td>Pre-Supplement</td>
<td>4.8 ± 2.2</td>
<td>4.7 ± 2.3</td>
<td>3.7 ± 2.2♦</td>
<td>3.2 ± 2.0†</td>
<td>2.7 ± 1.9†</td>
</tr>
<tr>
<td>Post-Supplement</td>
<td>4.7 ± 2.4</td>
<td>4.6 ± 2.5</td>
<td>3.6 ± 2.4♦</td>
<td>3.1 ± 2.2†</td>
<td>2.6 ± 1.8†</td>
</tr>
<tr>
<td><strong>50 Hz Peak Torque (Nm)</strong></td>
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<td></td>
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<tr>
<td>Pre-Supplement</td>
<td>10.1 ± 5.5</td>
<td>9.6 ± 4.8*</td>
<td>9.0 ± 4.5♦</td>
<td>8.5 ± 4.5†</td>
<td>8.5 ± 4.6†</td>
</tr>
<tr>
<td>Post-Supplement</td>
<td>9.8 ± 5.0</td>
<td>9.0 ± 4.4*</td>
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<td>8.2 ± 4.5†</td>
<td>7.8 ± 4.4†</td>
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<td><strong>10/50 Hz Torque Ratio</strong></td>
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<tr>
<td>Pre-Supplement</td>
<td>0.51 ± 0.14</td>
<td>0.52 ± 0.16</td>
<td>0.42 ± 0.16*</td>
<td>0.39 ± 0.16†</td>
<td>0.32 ± 0.15†</td>
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<tr>
<td>Post-Supplement</td>
<td>0.49 ± 0.11</td>
<td>0.51 ± 0.12</td>
<td>0.41 ± 0.13†</td>
<td>0.37 ± 0.14†</td>
<td>0.33 ± 0.12†</td>
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<tr>
<td><strong>TA EMG (µ Volts)</strong></td>
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<tr>
<td>Pre-Supplement</td>
<td>161.3 ± 62.9</td>
<td>139.2 ± 67.6*</td>
<td>128.6 ± 62.2†</td>
<td>110.5 ± 41.6†</td>
<td>102.4 ± 42.2†</td>
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<tr>
<td>Post-Supplement</td>
<td>141.1 ± 49.5</td>
<td>119.9 ± 46.3*</td>
<td>114.2 ± 43.8*</td>
<td>114.2 ± 43.8*</td>
<td>109.2 ± 47.1†</td>
</tr>
</tbody>
</table>

Numbers represent Mean ± Standard Deviation  
MVC: Maximal Voluntary Contraction  
TA EMG: Tibialis Anterior Electromyography  
* p<0.05 Compared to Baseline, † p<0.0001 Compared to Baseline
Table 4.2 (Continued): Pre-Supplement and Post-Supplement Measures for Fatigue Protocol and Recovery Period

<table>
<thead>
<tr>
<th></th>
<th>Recovery 2 min</th>
<th>Recovery 5 min</th>
<th>Recovery 10 min</th>
<th>Recovery 15 min</th>
<th>Recovery 20 min</th>
<th>48 Hour Recovery</th>
<th>96 Hour Recovery</th>
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<tbody>
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<tr>
<td>Pre-Supplement</td>
<td>15.6 ± 9.5†</td>
<td>17.4 ± 10.3†</td>
<td>17.8 ± 9.1†</td>
<td>17.8 ± 9.3†</td>
<td>17.9 ± 7.7†</td>
<td>22.3 ± 9.8†</td>
<td>22.3 ± 10.2†</td>
</tr>
<tr>
<td>Post-Supplement</td>
<td>14.0 ± 9.5†</td>
<td>15.3 ± 9.5*</td>
<td>15.8 ± 8.5*</td>
<td>15.3 ± 7.8*</td>
<td>16.4 ± 7.8*</td>
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<td>21.3 ± 9.4</td>
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<tr>
<td>Pre-Supplement</td>
<td>2.8 ± 1.8†</td>
<td>2.5 ± 1.8†</td>
<td>2.2 ± 1.4†</td>
<td>2.2 ± 1.5†</td>
<td>2.3 ± 1.5†</td>
<td>3.7 ± 2.1†</td>
<td>4.2 ± 2.4*</td>
</tr>
<tr>
<td>Post-Supplement</td>
<td>2.5 ± 1.7†</td>
<td>2.4 ± 1.7†</td>
<td>2.1 ± 1.3†</td>
<td>2.2 ± 1.3†</td>
<td>2.2 ± 1.2†</td>
<td>4.4 ± 1.9</td>
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<td><strong>50 Hz Peak Torque (Nm)</strong></td>
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<tr>
<td>Pre-Supplement</td>
<td>8.9 ± 4.9*</td>
<td>8.6 ± 5.2*</td>
<td>8.9 ± 5.2*</td>
<td>9.1 ± 5.0*</td>
<td>9.2 ± 5.0*</td>
<td>9.4 ± 4.7</td>
<td>9.9 ± 5.3</td>
</tr>
<tr>
<td>Post-Supplement</td>
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<td>8.1 ± 4.9*</td>
<td>8.2 ± 5.1*</td>
<td>8.3 ± 5.0*</td>
<td>8.4 ± 4.7*</td>
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<tr>
<td><strong>10/50 Hz Torque Ratio</strong></td>
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</tr>
<tr>
<td>Pre-Supplement</td>
<td>0.33 ± 0.15†</td>
<td>0.29 ± 0.12†</td>
<td>0.27 ± 0.11†</td>
<td>0.25 ± 0.11†</td>
<td>0.25 ± 0.11†</td>
<td>0.40 ± 0.12†</td>
<td>0.44 ± 0.14*</td>
</tr>
<tr>
<td>Post-Supplement</td>
<td>0.32 ± 0.12†</td>
<td>0.30 ± 0.12†</td>
<td>0.28 ± 0.12†</td>
<td>0.28 ± 0.11†</td>
<td>0.28 ± 0.12†</td>
<td>0.47 ± 0.11</td>
<td>0.46 ± 0.12</td>
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<tr>
<td><strong>TA EMG (µ Volts)</strong></td>
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</tr>
<tr>
<td>Pre-Supplement</td>
<td>99.0 ± 49.3†</td>
<td>107.6 ± 53.9†</td>
<td>111.1 ± 51.7†</td>
<td>112.5 ± 57.8†</td>
<td>115.2 ± 52.0†</td>
<td>139.8 ± 64.1*</td>
<td>139.3 ± 56.1*</td>
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<tr>
<td>Post-Supplement</td>
<td>99.0 ± 39.7†</td>
<td>105.1 ± 41.0†</td>
<td>107.3 ± 40.0†</td>
<td>111.9 ± 44.8*</td>
<td>114.2 ± 45.4°</td>
<td>134.5 ± 41.9</td>
<td>135.2 ± 47.3</td>
</tr>
</tbody>
</table>

Numbers represent Mean ± Standard Deviation  
MVC: Maximal Voluntary Contraction  
TA EMG: Tibialis Anterior Electromyography  
* p<0.05 Compared to Baseline, † p<0.0001 Compared to Baseline
Figure 4.1: MVC Torque Measurements for Pre and Post-Supplement Limbs

* p<.05 Compared to baseline  + p<.0001 Compared to baseline

Figure 4.2: 10 Hz Peak Torque Measurements for Pre and Post-Supplement Limbs

* p<.05 Compared to baseline  + p<.0001 Compared to baseline

45
Figure 4.3: 50 Hz Peak Torque Measurements for Pre and Post-Supplement Limbs

* p<.05 Compared to baseline  + p<.0001 Compared to baseline

Figure 4.4: 10/50 Hz Torque Ratio for Pre and Post-Supplement Limbs

* p<.05 Compared to baseline  + p<.0001 Compared to baseline
Figure 4.5: TA EMG for Pre and Post-Supplement Limbs

* p<.05 Compared to baseline       + p<.0001 Compared to baseline
Chapter V: Discussion

The primary purpose of this study was to determine if three weeks of HMB supplementation could attenuate the effects of LFF caused by eccentric muscle contractions of the TA muscle. The major finding of this study was that HMB did attenuate the effects of LFF caused by eccentric muscle contractions in the TA muscle. This positive finding was due to the results that demonstrated that the 10/50 Hz torque ratio, and 10 Hz peak torque in the HMB post-supplement limb recovered faster when compared to the pre-supplement limb. In addition, it was observed that the MVC torque in the post-supplement limb recovered faster when compared to the pre-supplement limb.

The 10/50 Hz torque ratio is a measure of LFF, and any significant reduction in the 10/50 Hz torque ratio following completion of a fatigue protocol, is interpreted as LFF (60). After the fatigue protocol, both the pre-supplement limb and the post-supplement limb had a significant relative 10/50 Hz torque ratio deficit (Figure 4.4). This reduction in the 10/50 Hz torque ratio indicated that the fatigue protocol used in this study, produced significant LFF. Also, the amount of change in the 10/50 Hz torque ratio that was observed in this study for the pre-supplement limb was similar to that found by Power et al. (60).

The 10/50 Hz torque ratio for the post-supplement limb recovered faster when compared to the pre-supplement limb (Figure 4.4). The post-supplement limb was recovered at the 48-hour recovery measurement time and the pre-supplement limb had not recovered at the 96-hour recovery measurement time. The current study supports the findings of Jones and colleagues (1989) which reported a torque ratio reduction for four days after an eccentric fatigue protocol (22). Jones et al. (1989) also reported that the torque ratio did not return to baseline levels until seven days after the eccentric fatigue protocol and that isometric and concentric contractions did not produce a reduced torque ratio (22). Power et al. (2010) reported a depressed torque ratio was present immediately after an eccentric fatigue protocol and was still present after 30 minutes of recovery (60). Since eccentric contractions have been reported to cause LFF and is slow to recover, it is possible that this is a consequence of some form of damage caused by the generation of high forces within the muscle (22). Based on the 10/50 Hz torque ratio, the pre-supplement limb was still showing significant LFF at the 96-hour recovery measurement time. In contrast, the post-supplement limb recovered faster,
and at the 48-hour recovery measurement time it was no longer showing significant signs of LFF. This is evidence that HMB supplementation positively affected the time course of LFF after the fatigue protocol. This positive affect from HMB supplementation may have been the result of reduced muscle damage in the post-supplement limb during the fatigue protocol or an increased recovery rate following the fatigue protocol. Since the current study did not employ any direct measure of muscle damage, it cannot be concluded which possible mechanism of action of HMB supplementation, may have occurred.

It has been suggested that HMB supplementation prevents muscle damage or regenerates damaged muscle cell membranes after activity (26, 43, 46, 53, 66, 88). The current study may be evidence that supports HMB’s ability to regenerate damaged muscle cell membranes. This is based on the faster recovery of the 10/50 Hz torque ratio and 10 Hz peak torque that was seen in the post-supplement limb in the current study. This faster regeneration of damaged muscle cell membranes has been suggested to be achieved by two mechanisms (26, 40, 50). The first mechanism involves conversion of HMB, in the cytosol of the liver, to β-hydroxy-β-methylglutarate-CoA (HMG-CoA) (26, 40, 50). HMG-CoA is then employed in cholesterol synthesis and could be rate limiting when cholesterol synthesis is in high demand, such as during periods of rapid cell growth or membrane repair that is seen after eccentric exercise (17). In addition, stressed or damaged muscle cells may not be able to make sufficient HMG-CoA to support adequate cholesterol synthesis for cell functions (41).

Supplemental HMB therefore, may be a source of HMG-CoA in damaged muscle cells to maintain adequate cholesterol synthesis and plasma membrane function (41). The second suggested mechanism contributing to the positive effects of HMB supplementation concerns HMB’s ability to be polymerized and covalently bonded with cell membrane structures to enhance membrane integrity (49, 50). This increased integrity may also be accomplished through the additional source of cholesterol provided by HMB supplementation (46). It has been hypothesized that the de novo synthesized cholesterol is used to support the cell membrane integrity, and thus assist in the prevention of muscle damage or used to regenerate damaged muscle cell membranes (26). Therefore, HMB supplementation may provide the necessary amount of HMG-CoA for cholesterol synthesis and membrane protection that would then result in decreased muscle damage and quicker recovery from resistance exercise (17). This is supported
by studies that have demonstrated that HMB supplementation has decreased markers of muscle damage following strenuous exercise (17, 26, 40, 55).

The 10 Hz peak torque represents low frequency muscle stimulation and in the current study it was used as another indicator of LFF. After the fatigue protocol, and following the 20-minute recovery period, both the pre-supplement limb and the post-supplement limb had a significant relative 10 Hz peak torque deficit (Figure 4.2). This supports the notion that LFF does not have a metabolic or ionic component. If a metabolic or ionic waste product accumulation contributed to the LFF, an increase in the 10 Hz peak torque toward baseline would be expected during the recovery period as seen in high frequency stimulation (i.e. 50 Hz) (32).

In the current study, based on the peak 10 Hz torque, there was still significant LFF observed in the pre-supplement limb 96 hours after the fatigue protocol (Figure 4.2). In contrast, the 10 Hz peak torque was recovered to non-significant levels in the post-supplement limb at 48 hours after the fatigue protocol. This is more evidence that HMB supplementation reduced the recovery time of LFF in the current study. This is because the recovery of the post-supplement limb at the 48-hour recovery measurement time is in contrast to the pre-supplement limb and other findings reported (22). Jones et al. (1989) reported that low frequency torque was reduced for the first four days after eccentric exercise, and did not recover until seven days after eccentric exercise (22).

The primary characteristic of LFF is the reduction of muscle force at low frequency stimulation (25). Motor unit discharge rates during voluntary skeletal muscle activation rarely exceed 30 Hz, so any voluntary muscle contraction would be greatly affected by LFF (25). This would include any strenuous activity, walking, stair climbing, and all other activities of daily living (25). This effect of LFF on voluntary muscle contraction could result in higher activation of the central nervous system, require the recruitment of a larger number of motor units or increased firing frequency, and cause greater feelings of effort during repetitive activities and may limit performance (25). The current study found that three weeks of HMB supplementation significantly reduced the slow recovery of LFF.

The 50 Hz peak torque represents high frequency muscle stimulation and in this study it was used as an indicator of high frequency fatigue. At the end of the fatigue protocol, both the pre-supplement limb and the post-supplement limb had a significant relative 50 Hz peak torque deficit (Figure 4.3). During the 20-minute recovery period the 50 Hz peak torque increased in both limbs. This recovery in the 50 Hz peak torque
indicated that high frequency fatigue recovered quickly and suggests that it has a metabolic or ionic basis. It has been previously reported that, the buildup of metabolic byproducts or ions from a fatigue protocol return toward resting levels during the early stages of the recovery period (32). As these byproducts are restored toward resting levels, the 50 Hz peak torque returns toward baseline levels (32). By the 48-hour recovery measurement time the 50 Hz peak torque had recovered to non-significant levels in both limbs. This is evidence that high frequency fatigue recovers faster than LFF. There were no differences between the pre-supplement and post-supplement limbs in the 50 Hz peak torque measurement during the fatigue protocol, or any recovery measurement times. Therefore, based on the 50 Hz peak torque measurement it is concluded that HMB supplementation had no effect on high frequency fatigue in the current study.

After the fatigue protocol the MVC torque was reduced by 50% in both the pre-supplement and the post-supplement limbs and both the pre-supplement and post-supplement limbs had a significant relative MVC torque deficit (Figure 4.1). This amount of reduction of force demonstrates that the fatigue protocol used in this study was intense enough to produce a sufficient amount of muscle fatigue. This study utilized a fatigue protocol adopted from Power et al. (2010) and achieved a greater degree of MVC torque reduction (60). The subjects in the Power et al. study had a MVC torque reduction to 72% of baseline after their fatigue protocol (60).

At the 20-minute recovery measurement time both the pre-supplement and post-supplement limbs had a significant relative MVC torque deficit (Figure 4.1). Due to the short time period of this fatigue, it is theorized that the cause of this fatigue is the buildup of some metabolite during the fatigue protocol. It has been reported that the concentration of inorganic phosphate (P_i) increases in the muscle fiber during muscle contractions (2). An increase in P_i in the muscle fiber reduces Ca^{2+} release and therefore reduces cross bridge cycling and force production (2). This type of fatigue can also be caused by a reduction in Ca^{2+} sensitivity (32). Reduced Ca^{2+} sensitivity is a condition where force at a given free Ca^{2+} concentration is lowered and it would take more Ca^{2+} release for the same amount of force production (32). The accumulation of P_i is also thought to reduce Ca^{2+} sensitivity in a fatiguing muscle (2). Since both the pre-supplement and post-supplement limbs were still significantly different from baseline, after 20 minutes of recovery, it is concluded that HMB supplementation had no effect on short term fatigue.
Muscle damage caused by eccentric muscle contractions has been documented directly by muscle biopsy samples and indirectly from losses in strength and range of motion, increases in muscle soreness, and increases in blood muscle proteins, such as creatine kinase (CK) and lactate dehydrogenase (LDH) (63). Among these markers, change in force generating capacity is considered to be the most reliable (30). The MVC torque in the post-supplement limb also recovered faster than the pre-supplement limb at the 48-hour and 96-hour recovery measurement times (Figure 4.1). This is an indicator that a degree of muscle damage was still present in the pre-supplement limb at the 96-hour recovery measurement time. Since the post-supplement limb’s MVC torque was nearly fully recovered after 48-hours of recovery, this study suggests that there was a positive effect of HMB supplementation on muscle damage recovery time after the fatigue protocol. Since both limbs were reduced to 50% of baseline after the fatigue protocol, it is thought that HMB supplementation decreased the recovery time of muscle damage, rather than influenced the amount of muscle damage that occurred in the post-supplement limb. An explanation of this reduced recovery time could be that HMB supplementation increased the repair or resynthesis process of the damaged proteins or structures that occurred in the eccentric fatigue protocol.

The current study showed that the MVC torque in the post-supplement limb recovered faster when compared to the pre-supplement limb. This could be explained by a positive effect of HMB supplementation but, it could also be a result of the baseline MVC torque being significantly different between the two limbs. At baseline the MVC torque for the post-supplement limb was 5.7 Nm less than the pre-supplement limb. Since the post-supplement limb’s baseline MVC torque was lower, it may have been easier and therefore faster for MVC torque to return to non-significant levels after the fatigue protocol. This may have contributed to the post-supplement limb’s faster recovery in the current study.

After the fatigue protocol, both the pre-supplement limb and the post-supplement limb had a significant relative TA EMG deficit which was still present after 20 minutes of recovery (Figure 4.5). This decrease in TA EMG may have been due to central fatigue, high frequency peripheral fatigue, or a combination of both factors. The post-supplement limb showed no relative TA EMG deficits at the 48-hour recovery measurement time and the pre-supplement limb still had relative deficits at the 48-hour and 96-hour recovery measurement times. This is evidence that HMB supplementation positively affected the recovery of the TA EMG in the current study. The current study is
in agreement with another study that saw a decrease in EMG activity in the forearm flexors after an eccentric fatigue protocol (89). In addition, it has been reported that EMG amplitude declines during maximal isometric contractions (23). This decline could be due to decreases in motor unit firing rates, neuromuscular propagation failure, or the slowing of conduction velocity (23). The current study is not in agreement with two other studies that saw increases in EMG activity in the hamstring muscle group (33) and the biceps brachii after an eccentric fatigue protocol (67). It was suggested that the increased EMG activity was due to greater recruitment of motor units and an increased motor unit discharge rate (67). The current study did not see an increase in EMG activity of the TA muscle after the fatigue protocol. This may be due to the increased central fatigue experienced by the subjects.

In conclusion the current study demonstrated that three weeks of HMB supplementation reduced the effects and time course of LFF after an eccentric fatigue protocol in the TA muscle. The 10/50 Hz torque ratio and the 10 Hz peak torque recovered faster in the post-supplement limb when compared to the pre-supplement limb. In addition, the MVC torque in the post-supplement limb may have recovered faster when compared to the pre-supplement limb. This study also supports the literature that there are two components of fatigue after an exercise bout, LFF that has a long time course lasting longer than 96 hours and a fast recovering high frequency fatigue. Since HMB supplementation has been shown to reduce LFF caused by eccentric muscle contractions it may provide quicker recovery from physical activity for recreational athletes, the general public, and people suffering from long term illnesses.
Chapter VI: Summary

Introduction

Muscular fatigue, defined as a failure to maintain a required or expected force/power for a given task, is a common result of physical activities ranging from athletic performance to activities of daily living (31). Low frequency fatigue (LFF) is muscular fatigue that occurs between 5 and 30 Hz stimulation frequency (34). The characteristics of LFF include decreased muscle force that is most severe at low stimulation frequencies, slow recovery of force that may take hours or days to resolve, and the presence of force decrements may remain in the absence of gross metabolic or electrical disturbance of the muscle (2, 3, 25, 34). This form of fatigue may be present following any form of intense exercise and is a large contributor to the feeling of muscle weakness after exercise (25, 84). The primary characteristic of LFF is the reduction of muscle force at low stimulation frequency and this reduction of force is typically greater than 50% of rested (baseline) values (25). Motor unit discharge rates during voluntary skeletal muscle activation rarely exceed 30 Hz, therefore, most voluntary muscle contractions may be substantially affected by LFF including any strenuous activity, walking, stair climbing, and all other activities of daily living (25).

It has been previously demonstrated that one of the causes of LFF is a reduction in the amount of calcium (Ca$^{2+}$) released from the sarcoplasmic reticulum (SR) (2). A decrease in Ca$^{2+}$ release affects muscle tension at low frequencies of stimulation to a much greater extent than muscle tension at high frequencies (25, 84). It seems likely that there is some degree of structural damage to one or more of the proteins or structures involved in excitation-contraction coupling (E-C coupling) and the long time course of LFF represents repair or re-synthesis of these proteins or structures (25, 84). In support of this muscle damage explanation it has been shown that LFF frequently occurs following eccentric exercises, and this LFF remains during the repair process if the muscle fiber was damaged during the eccentric exercise (25).

Eccentric muscle contractions produce more muscle damage than concentric or static contractions (2, 6, 61). These types of contractions are part of activities of daily living including walking downstairs or lowering a heavy weight. It is believed that the greater muscle damage caused by eccentric contractions is due to mechanical insult and not metabolic fatigue (6). In overstretched sarcomeres the sarcoplasmic reticulum could
be damaged or its connection with the transverse tubules (T-tubules) might be altered (2). Potential evidence for this is the reduction in force at low stimulation frequency following eccentric muscle contractions (6). This LFF has been found to be caused by E-C uncoupling and in particular a reduced amount of Ca$^{2+}$ release from the SR (2, 6, 61, 83).

Supporters of a nutritional supplement, β-Hydroxy-β-Methylbutyrate (HMB) supplementation, claim that HMB supplementation prevents muscle damage or enhances the regeneration of damaged muscle cell membranes. However, no research has evaluated the effect of HMB supplementation on low frequency fatigue. Therefore, the purpose of this study was to determine if three weeks of HMB supplementation could attenuate the effects of low frequency fatigue caused by eccentric muscle contractions of the tibialis anterior muscle. It was hypothesized that HMB supplementation would reduce LFF and enhance recovery after an eccentric exercise protocol. Therefore, this study investigated the effects of three weeks of HMB supplementation on neuromuscular performance, following fatiguing exercise, in healthy subjects.

**Research Questions and Hypotheses**

The first research question that this study sought to answer was: Are there different types of muscle fatigue after an eccentric fatigue protocol in the TA muscle? It was hypothesized that there would be two distinct types of fatigue after the eccentric fatigue protocol. This will include a high frequency fatigue, measured by the 50 Hz peak torque which will recover faster than the low frequency fatigue, measured by the 10 Hz peak torque. This hypothesis was based on prior research that showed that there were two groups of fatigue after activity and they were low frequency fatigue (LFF) at 5 to 30 Hz and high frequency fatigue at 50 to 100 Hz (35). In addition, high frequency fatigue recovers very quickly usually within minutes after stimulation or exercise has stopped and LFF where recovery of force is slow, taking hours or days (35).

The second research question that this study sought to answer was: Does HMB supplementation have any effect on the recovery time of LFF following eccentric exercise? It was hypothesized that HMB supplementation would reduce the recovery time of low frequency fatigue following eccentric exercise. The 10/50 Hz torque ratio and 10 Hz peak torque would recover toward baseline levels faster in the HMB supplemented limb. This hypothesis was based on prior research that reported that
HMB supplementation prevents muscle damage and/or aids in regenerating damaged muscle cell membranes (26, 43, 46, 53, 66, 88).

The final research question that this study sought to answer was: Does HMB supplementation have any effect on the recovery time of muscle damage caused by eccentric exercise? It was hypothesized that HMB supplementation would reduce the recovery time of muscle damage caused by eccentric exercise. As demonstrated by a faster return of MVC torque to baseline in the HMB supplemented limb. This hypothesis was based on prior research that reported that HMB supplementation imparted anticatabolic properties and reduced muscle damage based on reduced enzyme markers of muscle damage such as creatine phosphokinase (CK), lactate dehydrogenase (LDH), and 3-methylhistidine (3-MH) (17, 21, 55, 70). In addition, HMB was reported to be converted into β-hydroxy-β-methylglutarate-CoA (HMG-CoA) and employed in cholesterol synthesis (26, 40, 50). Without cholesterol, cells cannot experience normal growth or recovery (90). Supplemental HMB therefore, may be a source of HMG-CoA in damaged muscle cells to maintain adequate cholesterol synthesis and allow for maximal cell growth, function, and recovery (41, 71).

Discussion

The major finding of this study was that HMB did attenuate the effects of LFF caused by eccentric muscle contractions in the TA muscle. This positive finding was supported by the results that demonstrated that the 10/50 Hz torque ratio, and 10 Hz peak torque in the HMB post-supplement limb recovered faster when compared to the pre-supplement limb. In addition, it was observed that the MVC torque in the post-supplement limb recovered faster when compared to the pre-supplement limb. The current study may be evidence that supports HMB’s ability to regenerate damaged muscle cell membranes. This is based on the faster recovery of the 10/50 Hz torque ratio, 10 Hz peak torque and MVC torque that was seen in the post-supplement limb in the current study.

The 10/50 Hz torque ratio is a measure of LFF, and any significant reduction in the 10/50 Hz torque ratio following completion of a fatigue protocol, is interpreted as LFF (60). The 10/50 Hz torque ratio for the post-supplement limb recovered faster when compared to the pre-supplement limb (Figure 4.4). The post-supplement limb was recovered at the 48-hour recovery measurement time and the pre-supplement limb was not recovered at the 96-hour recovery measurement time. Based on the 10/50 Hz
torque ratio, the pre-supplement limb was still showing significant LFF at the 96-hour recovery measurement time. In contrast, the post-supplement limb recovered faster, and at the 48-hour recovery measurement time it was no longer showing significant signs of LFF. This is evidence that HMB supplementation positively affected the time course of LFF after the fatigue protocol.

The 10 Hz peak torque represents low frequency muscle stimulation and in the current study it was used as another indicator of LFF. After the fatigue protocol, and following the 20-minute recovery period, both the pre-supplement limb and the post-supplement limb had a significant relative 10 Hz peak torque deficit (Figure 4.2). In the current study, based on the peak 10 Hz torque, there was still significant LFF observed in the pre-supplement limb 96 hours after the fatigue protocol (Figure 4.2). In contrast, the 10 Hz peak torque was recovered to non-significant levels in the post-supplement limb at 48 hours after the fatigue protocol. This is more evidence that HMB supplementation reduced the recovery time of LFF in the current study.

Based on these findings HMB supplementation may be beneficial to athletes, recreational athletes, elderly, and anyone who participates in physical activity. This supplement would be most beneficial to people who experience high levels of muscle damage and LFF during physical activity. This would include sedentary people starting a physical activity program or active people changing their physical activity program. In these cases the potential for muscle damage and LFF is high and HMB supplementation may help these individuals recover more quickly from physical activity. This supplement would not be beneficial to individuals who are active and not changing their programs. This is because the potential for muscle damage and LFF would be low and HMB supplementation would not help these individuals recover from physical activity faster.

The 50 Hz peak torque represents high frequency muscle stimulation and in this study it was used as an indicator of high frequency fatigue. At the end of the fatigue protocol, both the pre-supplement limb and the post-supplement limb had a significant relative 50 Hz peak torque deficit (Figure 4.3). During the 20-minute recovery period the 50 Hz peak torque increased in both limbs. By the 48-hour recovery measurement time the 50 Hz peak torque had recovered to non-significant levels in both limbs. This is evidence that high frequency fatigue recovers faster than LFF. There were no differences between the pre-supplement and post-supplement limbs in the 50 Hz peak torque measurement during the fatigue protocol, or any recovery measurement times. Therefore, based on the 50 Hz peak torque measurement it is concluded that HMB
supplementation had no effect on high frequency fatigue in the current study. At the 20-minute recovery measurement time both the pre-supplement and post-supplement limbs had a significant relative MVC torque deficit (Figure 4.1). Since both the pre-supplement and post-supplement limbs were still significantly different from baseline, after 20 minutes of recovery, it is concluded that HMB supplementation had no effect on short term muscle fatigue. Future research should focus on nutritional supplements that enhance performance and reduce the amount of force loss during a fatiguing protocol. If such a supplement can be found then high frequency fatigue and force loss would be reduced during physical activity and performance would increase.

The MVC torque in the post-supplement limb also recovered faster than the pre-supplement limb at the 48-hour and 96-hour recovery measurement times (Figure 4.1). This is an indicator that a degree of muscle damage was still present in the pre-supplement limb at the 96-hour recovery measurement time. Since the post-supplement limb’s MVC torque was nearly fully recovered after 48-hours of recovery, this study suggests that there was a positive effect of HMB supplementation on muscle damage during the recovery time after the fatigue protocol. An explanation of this reduced recovery time could be that HMB supplementation increased the repair or resynthesis process of the damaged proteins or structures that occurred during the eccentric fatigue protocol. This is more support that HMB supplementation may be beneficial to individuals who experience high levels of muscle damage during their physical activity.

The current study showed that the MVC torque in the post-supplement limb recovered faster when compared to the pre-supplement limb. This could be explained by a positive effect of HMB supplementation but, it could also be a result of the baseline MVC torque being significantly different between the two limbs. At baseline the MVC torque for the post-supplement limb was 5.7 Nm less than the pre-supplement limb. Since the post-supplement limb’s baseline MVC torque was lower, it may have been easier for it to return to non-significant levels after the fatigue protocol. This may have contributed to the post-supplement limb’s faster recovery in the current study.

**Conclusion**

The current study demonstrated that three weeks of HMB supplementation reduced the effects and time course of LFF after an eccentric fatigue protocol in the TA muscle. The 10/50 Hz torque ratio, and the 10 Hz peak torque recovered faster in the post-supplement limb when compared to the pre-supplement limb. In addition, the MVC
torque in the post-supplement limb may have recovered faster when compared to the pre-supplement limb. This study also supports the literature that there are two components of fatigue after an exercise bout, LFF that has a long time course, lasting longer than 96 hours, and a fast recovering high frequency fatigue. Since HMB supplementation has been shown to reduce LFF caused by eccentric muscle contractions it may provide quicker recovery from physical activity for recreational athletes, the general public, and people suffering from long term illnesses.

Future Research

The TA muscle used in this study is not a highly trained muscle and the subjects used were only recreationally active. So, future research should be focused on HMB’s ability to reduce the effects of LFF in other muscles and different populations, such as highly trained, non-trained, and older populations. The quadriceps muscle group would be a good muscle for future research in HMB’s ability to positively effect LFF and muscle damage. This is because the quadriceps muscle group is a prime mover in many activities and sporting events. Also, for the elderly population the quadriceps are the main muscles in rising from a chair and locomotion.

Full training studies with HMB supplementation should be conducted to assess its effects on the whole body during an exercise program. Since the current study potentially supports HMB’s ability to increase muscle recovery from muscle damage, the training studies should be designed to produce muscle damage. This could be done by using a sedentary population starting a training program or a trained population that would be drastically changing their program. A reason that some training studies did not see any benefit of HMB supplementation could be because the training itself did not cause significant muscle damage and LFF in the subjects used. In addition, these studies should be of varying lengths to test HMB’s effects on reducing muscle damage over different periods of time.

The current study supports HMB’s ability to reduce muscle damage but, no direct measures of muscle damage were used in this study. So, future research, involving HMB, should utilize some form of direct measure of muscle damage. This could include muscle biopsies or measures of plasma proteins such as creatine phosphokinase, lactate dehydrogenase or 3-methylhistidine. In these types of studies it could be shown if HMB supplementation improves protein synthesis or decreases protein breakdown after physical activity.
References


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