2015

The Effects of Four Weeks of High Intensity Interval Training and β-hydroxy-β-methylbutyric Free Acid on the Onset of Neuromuscular Fatigue

Amelia Miramonti
University of Central Florida

THE EFFECTS OF FOUR WEEKS OF HIGH INTENSITY INTERVAL TRAINING AND β-HYDROXY-β-METHYLBUTYRIC FREE ACID SUPPLEMENTATION ON THE ONSET OF NEUROMUSCULAR FATIGUE

by

AMELIA ANNE MIRAMONTI
B.S. University of Central Florida, 2013

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Educational and Human Sciences
in the College of Education and Human Performance
at the University of Central Florida
Orlando, FL

Spring Term
2015

ABSTRACT

This study investigated the effects of high intensity interval training (HIIT) and β-hydroxy-β-methylbutyric free acid (HMB_{FA}) on the onset of neuromuscular fatigue in healthy young men and women. Thirty-seven subjects (22 men and 15 women; mean ± SD age = 22.8 ± 3.4yrs) completed an incremental cycle ergometer test (GXT) to exhaustion. During the GXT, electromyography (EMG) signals from the right vastus lateralis were recorded to determine the power output at the onset of neuromuscular fatigue (PWC_{FT}), and peak wattage was used to assign individual training loads.

After baseline testing (PRE), subjects were randomly assigned to one of three groups: control (C, n = 9), training with placebo (P, n = 14), or training with supplementation (S, n = 14). Subjects assigned to P and S completed 12 HIIT sessions over 4 weeks while subjects assigned to C were asked to maintain their normal diet and activity patterns. After 4 weeks, subjects returned for post-testing (POST). The PWC_{FT} values (W) were determined using a D_{MAX} method. The EMG amplitude root mean square (μVrms) values were used to generate a third-order polynomial regression (3PR) representing the increase in μVrms versus time of the GXT. The onset of fatigue (T_F) was defined as the x-value (time, s) of the point on the 3PR that measured the maximal perpendicular distance from the line between the first and last data points. T_F was used to estimate PWC_{FT} according to the equation: $PWC_{FT} = P_O + a \cdot (n/N)$, where P_O is the power output of the stage in which T_F occurred, a is the increment in power output between GXT stages (25W), n is the difference (s) between T_F and the beginning of the stage during which T_F occurred, and N is the duration of a stage (120s).

A two-way repeated measures ANOVA was used to identify group × time interaction for PWC_{FT}. If a significant interaction occurred, one-way factorial ANOVAs were used. Fisher's least significant difference post hoc comparisons were performed between groups. If a significant main effect occurred, dependent samples t-tests with Bonferroni corrections ($p = [0.05/3] = 0.017$) were performed across time for each group.

The two-way ANOVA resulted in a significant interaction ($F = 6.69$, $p = 0.004$). Follow-up analysis with one-way ANOVA resulted in no difference among groups at PRE ($F = 0.87$, $p = 0.43$), however a significant difference was shown for POST values ($F = 5.46$, $p = 0.009$). Post-hoc analysis among POST values showed significant differences between S and both P ($p = 0.034$) and C ($p = 0.003$). No differences ($p = 0.226$) were noted between P and C. Paired samples t-tests detected significant changes following HIIT for S ($p < 0.001$) and P ($p = 0.016$), but no change in C ($p = 0.473$).

Results of this study indicate that HIIT was effective in delaying the onset of fatigue, but supplementation with HMB_{FA} in conjunction with HIIT was more effective than HIIT alone. An increase in PWC_{FT} represents an increase in the maximal power output an individual can sustain without eliciting fatigue. Therefore, HIIT can be used to improve performance in both endurance activities as well as intermittent sports. In addition, HMB_{FA} supplementation is a simple method that can be used to maximize the benefits of HIIT.

Acknowledgments: Metabolic Technologies, Inc. provided the supplement and funding for this study.

This manuscript is dedicated to all those who have helped me, in ways both large and small, on my roundabout way of getting to where I am today.

ACKNOWLEDGMENTS

I cannot convey how grateful I am for the patience and guidance of my thesis committee members, the helpfulness of my fellow graduate students and friends, and the support of my friends and family throughout this entire process.

I would like to extend my greatest thanks to my advisor, Dr. Jeffrey Stout, without whom this project never would have been started – let alone finished. Although it was a long process, Dr. Stout helped to guide me and keep me focused, the latter being a near-Sisyphean task. Dr. Stout also had the patience to deal with my somewhat obsessive-yet-procrastinating personality.

I would also like to thank both Dr. Stout and Dr. Jay Hoffman for accepting me into the graduate program and offering me the tremendous opportunity to work as a graduate assistant; this has been the greatest experience for me and I sincerely appreciate it. My gratitude also goes out to Dr. David Fukuda, who I pestered endlessly with questions about calculus, statistics, computer programs, and whatever else popped in to my head. I also want to thank him for the many times he had to edit my numerous drafts and let me know where I was being unclear or may have an error.

Another huge thanks to Dr. Ned Robinson, without whom I would not have had data to analyze in the first place, and would never have been able to complete my reliability testing! He was also an invaluable source of information and insight throughout analysis and writing processes.

Which brings me to my next round of thanks – to all the other graduate students (current and former) who were involved in data collection and/or participated in the study: Dr. Jerry Mangine, Dr. Adam Wells, Ran Wang, Jeremy Townsend, Adam Jajtner, Michael La Monica, Jonathan Bohner, Dani Reardon, Kayla Baker, and Joshua Riffe. In addition, I would like to thank all of the other current & former graduate students in the lab who may have helped with training, helped me with editing, or just helped keep me sane throughout these last two years: Dr. Bill McCormack, Dr. Adam Gonzalez, Kyle Beyer, Carleigh Boone, Gabe Pruna, Mattan Hoffman, Dave Church, Alyssa Varanoske, and Sandro Bartolomei.

In particular I would like to thank Michael La Monica, who is probably the only person I bothered as much (or more) than Dr. Stout and Dr. Fukuda, and who was always there to answer my questions about the thesis process or read over a sentence/ paragraph/draft before I sent it off to the professors.

I would also like to thank the administrative staff at UCF, including Yaffa Hoffman, who hosted and fed us 'orphan' graduate students at holidays and gave advice on anything you might need, Laura Wilcox, who helped with each step of the thesis process, and others too numerous to list.

Lastly, (but certainly not least!) I would like to thank my friends and family who have been immensely supportive, doing everything from giving me invaluable transportation (Grandma Miramonti) or advice on data analysis (Dad), and keeping me clothed and fed (Mom), to keeping my spirits and confidence up (Nandini & Nicole) reminding me to maintain some balance and have some fun (Maria, Kristen, & Laurel), or just cheering me on no matter what (Grandma and Grandpa Butson).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

3PR – Third-order polynomial regression

C – Control group

D_{MAX} – Maximal perpendicular distance

EMG – Electromyography

GXT – Graded exercise test

HIIT – High intensity interval training

HMB – β-Hydroxy-β-methylbutyrate

HMB_{Ca} – Calcium salt of β-hydroxy-β-methylbutyrate

HMB_{FA} – β-Hydroxy-β-methylbutyric free acid

P – Training group with placebo

PRE – Baseline testing

POST – Post testing

PWC_{FT} – Physical working capacity at neuromuscular fatigue threshold

S – Training group with supplement (HMB)

T_F – Time of onset of fatigue

$\dot{V}O_{2MAX}$ – Maximal oxygen consumption

$\dot{V}O_{2PEAK}$ – Peak oxygen consumption

CHAPTER I: INTRODUCTION

Recently, high intensity interval training (HIIT) has gained popularity as both an effective and efficient training modality for many populations. In general, HIIT involves short bouts of high intensity exercise interspersed with brief periods of low intensity exercise or rest (Billat, 2001; Buchheit & Laursen, 2013a; Buchheit & Laursen, 2013b; Laursen & Jenkins, 2002). In comparison to traditional aerobic endurance training, HIIT has been shown to elicit similar-magnitude improvements in aerobic capacity despite lower total training volume and exercise time (Burgomaster et al., 2008; Gibala et al., 2006). Protocol specifics (work-to-rest ratios, number of intervals, and relative intensity of work and rest intervals) vary widely and it has been suggested that specific adaptations may be maximized by modifying these factors (Buchheit & Laursen, 2013b; Clark, West, Reynolds, Murray, & Pettitt, 2013). This is supported by the discernable differences in specific muscular adaptations observed following sprint training compared to speed endurance training (Mohr et al., 2007).

Daussin and colleagues (2008) compared continuous and interval training protocols with similar total exercise duration and volume in sedentary subjects and found that improvements in maximal aerobic capacity following continuous training were mainly the result of peripheral adaptations (increased capillary density and increased arteriovenous difference), whereas the improvements following interval training resulted from both peripheral (increased arteriovenous difference) and central adaptations (increased cardiac output); on the other hand, interval training did not appear to increase capillary density. However, Laursen and colleagues (2005) demonstrated that

improvements in trained athletes following HIIT may be primarily due to peripheral adaptations. Edge and colleagues (2006) also compared volume-matched continuous and interval training using recreationally active women and found that while increases in $\dot{V}O_{2PEAK}$ and lactate threshold (LT) were similar between groups, only interval training increased muscle buffering capacity. Additionally, HIIT is associated with increased efficiency of skeletal muscle substrate utilization via increases in storage, transport, and enzyme content (Perry, Heigenhauser, Bonen, & Spriet, 2008; Talanian, Galloway, Heigenhauser, Bonen, & Spriet, 2007) and improvements in measures of neuromuscular fatigue (Smith, Moon et al., 2009). Overall, HIIT has been shown to be effective in both trained and untrained populations following relatively short training protocols that involve a relatively small time commitment on a weekly basis. However, the varied adaptations in response to specific HIIT protocols must be considered when comparing HIIT studies with large differences in protocol specifics. In addition, changes in measures of central and peripheral fatigue may differ depending on the HIIT protocol used. As a result, responses to HIIT should be assessed using a variety of methods.

The branched-chain amino acid, leucine, has been associated with increases in muscle protein synthesis (Anthony, Anthony, Kimball, & Jefferson, 2001; Koopman et al., 2005), mitochondrial biogenesis and fatty acid oxidation (Sun & Zemel, 2009), decreases in muscle protein breakdown via the ubiquitin pathway (Baptista et al., 2010), and may contribute to cholesterol synthesis (Nissen & Abumrad, 1997). It has been suggested that some of the benefits of leucine supplementation occur via one of its metabolites, β-hydroxy-β-methylbutyrate (HMB) (Holecek, Muthny, Kovarik, & Sispera,

2009; G. J. Slater & Jenkins, 2000; G. J. Wilson, Wilson, & Manninen, 2008). In the body, approximately 5% of leucine is converted to HMB (Van Koevering & Nissen, 1992). A typical protein supplement contains 1-8-2.3g of leucine (Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009) which would be equivalent to approximately 9-12 mg of HMB. However, in studies investigating the benefits of HMB supplementation a typical dose is 3 grams per day; in order to achieve an equivalent amount of HMB in the body one would need to ingest approximately 60 grams of leucine or over 600 grams of high quality protein (J. M. Wilson et al., 2013). Therefore, supplementation with HMB may be a more efficient method to maximize the desired benefits than ingestion of either leucine or a protein supplement.

In some investigations, supplementation with HMB has been shown to be beneficial in increasing strength (Panton, Rathmacher, Baier, & Nissen, 2000), improving muscle protein balance (Nissen et al., 1996; Wilkinson et al., 2013), and attenuating markers of muscle damage after heavy resistance training (Hoffman et al., 2010; Nissen et al., 1996; Panton et al., 2000; Townsend et al., 2013; J. M. Wilson, Lowery et al., 2013), although others have failed to see these effects (Gonzalez et al., 2014; G. Slater et al., 2001). While much of the research has investigated the effects of HMB supplementation when combined with resistance training, it has also been shown to be beneficial for endurance athletes (Knitter, Panton, Rathmacher, Petersen, & Sharp, 2000; Vukovich & Dreifort, 2001). Knitter and colleagues (2000) tested the effects of 6 weeks of 3 grams per day of HMB on markers of muscle damage in trained individuals following a prolonged run and found that HMB attenuated increases in creatine

phosphokinase and lactate dehydrogenase, but reported no differences in performance. These results support the hypothesis that HMB attenuates muscle damage or improves recovery from damaging exercise. Vukovich and Dreifort (2001), on the other hand, measured changes in performance-related measures, such as $\dot{V}O_{2PEAK}$ and the onset of blood lactate accumulation (OBLA), following 14-day supplementation protocols in trained cyclists and reported increased OBLA and time to reach $\dot{V}O_{2PEAK}$. However, not all studies have shown similar results; O'Connor and Crowe (2003) reported no effects on aerobic or anaerobic measures in elite male rugby players following 6 weeks of supplementation with HMB.

In general, HMB supplementation has been shown to be safe in humans (Nissen et al., 2000). Currently there are two forms of supplemental HMB available: the monohydrated calcium salt (HMB_{Ca}) and the free acid gel form (HMB_{FA}). HMB_{Ca} has been in use longer and is the form that has been studied more extensively; however, a dose of HMB_{FA} results in a shorter time to peak plasma concentration following ingestion, a higher peak plasma concentration, and a greater area under the curve than an equivalent dose of HMB_{Ca} (Fuller, Sharp, Angus, Baier, & Rathmacher, 2011). Therefore, the form of HMB used could impact the consistency of results when comparing between studies, even if the dosage is equivalent.

More recently, HMB has been used in conjunction with HIIT, using both running (Lamboley, Royer, & Dionne, 2007) and cycling protocols (Robinson IV et al., 2014). Lamboley and colleagues (2007) investigated the effects of 5 weeks of 3 grams per day of HMB_{Ca} and 3 days per week of running HIIT on aerobic performance measures in

recreationally active college students. While both the HMB and placebo groups improved $\dot{V}O_{2MAX}$, VT, and RCP, greater improvements in $\dot{V}O_{2MAX}$ and RCP were seen in the HMB-supplemented group compared to placebo. These results support the hypothesis that HMB may be more effective in less trained subjects, as increases in aerobic capacity were not seen in the investigations using trained subjects (Knitter et al., 2000; O'Connor & Crowe, 2003; Vukovich & Dreifort, 2001). Robinson and colleagues (2014) also used untrained subjects, and reported significantly greater increases in $\dot{V}O_{2PEAK}$, VT, and power at VT in the supplemented group compared to placebo after 4 weeks of 3 days per week of cycle ergometer based HIIT with 3 grams per day of HMB_{FA} or a placebo.

Only two studies have investigated the effects of HIIT with HMB supplementation, and both investigated changes in aerobic capacity and fatigue thresholds based on gas exchange analysis (Lamboley et al., 2007; Robinson IV et al., 2014). Another common, noninvasive method for examining fatigue is through the use of electromyography (EMG) based measures of peripheral fatigue. Currently, only one study has investigated changes in neuromuscular fatigue thresholds following HIIT (Smith et al., 2009), and to our knowledge, none have investigated the effects of HMB on neuromuscular fatigue.

Electromyography based measures of fatigue often use changes in the amplitude and/or frequency domains of the signal to determine the onset of fatigue and demarcate the moderate, heavy, and severe exercise intensity domains. Two methods, physical working capacity fatigue threshold (PWC_{FT}) and electromyographic fatigue threshold

(EMG_{FT}), define the onset of neuromuscular fatigue as a significant increase in EMG amplitude during exercise, indicating the recruitment of additional motor units and the increased frequency of motor unit activation (Bergstrom et al., 2011; Camic et al., 2010; Camic et al., 2014; deVries, Moritani, Nagata, & Magnussen, 1982; deVries et al., 1987; deVries et al., 1989; deVries et al., 1990; Graef et al., 2008; Housh, deVries, Johnson, & Evans, 1991; Kendall et al., 2010). While PWC_{FT} and EMG_{FT} share a common underlying physiological mechanism, PWC_{FT} is typically determined using submaximal loads whereas EMG_{FT} is generally determined using maximal to supramaximal discontinuous work bouts. Although the method of determination differs, PWC_{FT} and EMG_{FT} are effectively analogous thresholds (Pavlat, Housh, Johnson, Schmidt, & Eckerson, 1993), to the extent that two of the authors of the original studies utilizing these techniques have used the terms interchangeably (deVries et al., 1987; Moritani & Yoshitake, 1998). Indeed, deVries and colleagues (1987) suggested that "the identification of PWC_{FT} is not seriously influenced by differences in the protocol leading to the fatigue threshold."

Both EMG_{FT} and PWC_{FT} have previously been used to assess changes following periods of supplementation and/or training: Stout and colleagues (2006) analyzed changes in PWC_{FT} following 28 days of supplementation with either creatine, β-alanine, or a combination, while Smith and colleagues (Smith et al., 2009) used EMG_{FT} to determine changes following three and six weeks of β-alanine supplementation and a fractal periodized HIIT protocol with undulating progression, ranging from 90-115% of the peak power output during the $\dot{V}O_{2PEAK}$ test. While the supplement investigated by

Smith and colleagues (2009) is different from that in the present study, they demonstrated that the HIIT protocol used was sufficient to stimulate adaptations that affect the onset of neuromuscular fatigue after a relatively short intervention.

The purpose of this study, therefore, was to examine the effects of 28 days of cycle ergometer based HIIT and HMB_{FA} supplementation on neuromuscular fatigue as measured using PWC_{FT} in untrained, college-aged men and women.

Purpose

1. To determine PWC_{FT} using a D_{MAX} method.
2. To determine the effects of four weeks of HIIT and HMB_{FA} supplementation on PWC_{FT}.

Research Questions

1. Does four weeks of HIIT increase PWC_{FT}?
2. Does HIIT with HMB_{FA} supplementation increase PWC_{FT} more than HIIT alone?

Hypotheses

1. HIIT will increase PWC_{FT}.
2. HIIT with HMB_{FA} will increase PWC_{FT} more than HIIT alone.

Operational Definitions

1. High Intensity Interval Training (HIIT) – Training comprised of short intervals of high intensity exercise interspersed with rest periods.

2. Physical Working Capacity at Neuromuscular Fatigue Threshold (PWC_{FT}) – The power output at the onset of neuromuscular fatigue, determined using a D_{MAX} method.
3. Maximal Perpendicular Distance (D_{MAX}) – A method for determining the point of inflection on a polynomial regression by finding the maximal perpendicular distance between the regression curve and a line between the first and last points used in the regression.

Delimitations

Forty men and women between the ages of 18 and 35 were recruited for this study. All participants completed a Confidential Medical and Activity History Questionnaire, Physical Activity Readiness Questionnaire, and a written informed consent prior to testing. The New England Institutional Review Board approved this research study. To be included in this study all participants were healthy and free of disease or injury. Also, participants were recreationally active with a minimum $\dot{V}O_{2PEAK}$ of $35ml \cdot kg^{-1} \cdot min^{-1}$ for men and $30ml \cdot kg^{-1} \cdot min^{-1}$ for women. Participants were asked to maintain their normal diet while enrolled in the study.

Assumptions

Theoretical Assumptions

1. Participants answered the Confidential Medical and Activity Questionnaire and Physical Activity Readiness Questionnaire accurately and truthfully.
2. All subjects gave maximal effort when performing the $\dot{V}O_{2PEAK}$ test.
3. Participants consumed a similar diet prior to each experimental testing session.

4. Participants were well-rested prior to each experimental testing session.
5. Participants abstained from all other supplements was maintained throughout the testing and training period.
6. Participants gave a maximal effort during each training session.
7. Participants were compliant with the supplementation protocol.
8. Participants were unable to identify whether they were taking the supplement or the placebo.

Statistical Assumptions

1. The population from which the samples are drawn is normally distributed.
2. The sample was randomly selected and the treatment order was randomly assigned.
3. The data met the assumption of sphericity. This requires that the repeated measures data demonstrate both homogeneity of variance and homogeneity of covariance.

Limitations

1. Due to the intensity of the exercise involved and the length of the study, participant withdrawal was inevitable.
2. The main recruiting mechanism of recruitment was in-class announcements through the College of Education and Human Performance courses, subject selection not truly random, which could affect internal validity and may impact generalizability.
3. The sample was made up of volunteers, therefore, not meeting the underlying assumptions of random selection.

CHAPTER II: LITERATURE REVIEW

In general, high intensity interval training (HIIT) refers to exercise programs consisting of repeated short to moderate duration bouts of exercise intensities greater than the anaerobic threshold, interspersed with brief periods of low intensity or passive rest (Billat, 2001; Buchheit & Laursen, 2013b). This type of training is designed to cause repeated physiological stress at a sufficiently high intensity to elicit chronic adaptations such as improved metabolic and energy efficiency, and to do so significantly faster and to a greater extent than traditional constant rate aerobic training (Jenkins & Quigley, 1993; Laursen et al., 2005).

It has been suggested that the metabolite of leucine, β-hydroxy-β-methylbutyrate (HMB) may mediate enhancement of some of the benefits of aerobic training (Knitter et al., 2000; Lamboley et al., 2007; Robinson IV et al., 2014; Vukovich & Dreifort, 2001). Knitter and colleagues (2000) proposed that HMB_{Ca} may improve intense aerobic training by attenuating skeletal muscle damage and accelerating recovery between training bouts leading to greater chronic adaptations. In support, Vukovich and Dreifort (2001) used a crossover design to examine the effects of 3 grams of HMB_{Ca} or placebo per day for 14 days in elite cyclists while average training volume was 300 miles per week. Only following the HMB_{Ca} supplementation period did the cyclists demonstrate a significant increase in peak oxygen consumption rate ($\dot{V}O_{2PEAK}$), and an increase in the onset of blood lactate accumulation (OBLA) during a graded exercise test. Further, Lamboley and colleagues (2007) examined the effect of 5 weeks of HMB_{Ca} supplementation and treadmill based HIIT in physically-active college students.

Specifically, they measured changes in $\dot{V}O_{2MAX}$, ventilatory threshold (VT), and respiratory compensation point (RCP) during a graded exercise test at baseline and following training. The participants completed 5 weeks of running HIIT, training 3 times per week at 1% grade; during this training period participants also supplemented with 3 grams per day of either HMB_{Ca} or a placebo. At post testing both HIIT groups demonstrated significant increases in $\dot{V}O_{2MAX}$, VT and RCP; however, the group that supplemented with HMB_{Ca} showed a 19% to 45% greater increase in all metabolic variables. Currently, research suggests that HMB_{Ca} supplementation in conjunction with endurance training may improve maximal and submaximal performance, not only by attenuating protein breakdown, but also by potentially augmenting mitochondrial protein synthesis for greater oxidative energy capacity (Knitter et al., 2000; Lamboley et al., 2007; Vukovich & Dreifort, 2001).

Surface electromyography is a noninvasive option for assessing the onset neuromuscular fatigue which has practical applications for evaluating individuals at baseline and after interventions to examine the effectiveness of various supplements (Camic et al., 2010; Housh, Johnson, Evans, & McDowell, 1991; McCormack et al., 2013; Stout et al., 2006; Stout et al., 2007) or training methods (deVries et al., 1989; Emerson, 2014; Smith et al., 2009) on delaying neuromuscular fatigue. There are several methods for analyzing this type of data, including those relevant to the current investigation: electromyographic fatigue threshold (EMG_{FT}) and physical working capacity at neuromuscular fatigue threshold (PWC_{FT}). Although EMG_{FT} is determined using a different method than PWC_{FT}, they are theoretically analogous thresholds, as both

determine the highest power output that can be sustained without resulting in a significant increase in EMG amplitude over time (Pavlat et al., 1993). Therefore, it is relevant to consider studies examining changes in EMG_{FT} when discussing the use of PWC_{FT} in similar applications.

Comparison of High Intensity Interval Training and Traditional Endurance Training

Helgerud, Høydal, Wang, Karlsen, Berg, Bjerkaas, Simonsen, Helgesen, Hjorth, Bach, Hoff (2007)

Aerobic High-Intensity Intervals Improve $\dot{V}O_{2MAX}$ More Than Moderate Training

The primary purpose of this study was to compare the effects of aerobic endurance training at different intensities and with different methods matched for total work and frequency. To measure these responses, $\dot{V}O_{2MAX}$, stroke volume, blood volume, lactate threshold, and running economy were examined. For this study, 40 healthy, nonsmoking, moderately trained male subjects were randomly assigned to one of four groups. The first group performed a continuous run at 70% heart rate max for 45 min. The second group performed a continuous run at lactate threshold which was set at 85% heart rate max for 24.25 min. The third group, an interval run group, performed 47 repetitions of 15-s intervals at 90– 95% heart rate max with 15 s of active rest at 70% heart rate max between each sprint. The final group trained a 4x4-min interval training at 90–95% heart rate max with 3 min of active rest at 70% heart rate max between each interval. All four training protocols were performed 3 times per week for 8 weeks. HIIT resulted in significantly increased $\dot{V}O_{2MAX}$ compared with continuous training intensities. The percentage increases for the 15/15 and 4 × 4 min groups were 5.5 and 7.2%,

respectively, reflecting increases in $\dot{V}O_{2MAX}$ from 60.5 to 64.4 $mL \cdot kg^{-1} \cdot min^{-1}$ and 55.5 to 60.4 $mL \cdot kg^{-1} \cdot min^{-1}$. Stroke volume also increased significantly by approximately 10% after interval training. The main finding of this study is that HIIT is significantly more effective than training at lactate threshold or continuous exercise even when performing the same total work for improving $\dot{V}O_{2MAX}$. The authors also state that improvements in $\dot{V}O_{2MAX}$ seem to be dependent on initial fitness level and type of exercise normally performed by the individual; those who performed short, intense, burst-style exercise, similar to football, saw no change in $\dot{V}O_{2MAX}$, while youth soccer players saw a 5-10% increase, and untrained individuals saw a 17.9% increase. The fact that stroke volume also increased significantly in the HIIT group leads the authors to suggest that stroke volume, as a component of cardiac output, is a key component of cardiorespiratory gains seen with this training regimen.

Daussin, Zoll, Dufour, Ponsot, Lonsdorfer-Wolf, Doutreleau, Mettauer, Piquard, Geny, Richard (2008)

Effect of interval versus continuous training on cardiorespiratory and mitochondrial functions: relationship to aerobic performance improvements in sedentary subjects

The goal of the study was to determine the effects of continuous training vs. HIIT yielding identical mechanical work and training duration on skeletal muscle and cardiorespiratory adaptations in sedentary subjects. Eleven subjects (6 men and 5 women) were randomly assigned to one of two 8-wk training programs in a cross-over design, separated by 12 weeks of detraining. $\dot{V}O_{2MAX}$ increased 9% after continuous

training and 15% with HIIT), whereas only HIIT was associated with faster $\dot{V}O_2$ kinetics (τ: 68.0 ± 1.6 vs. 54.9 ± 0.7 s, $P < 0.05$) measured during a test to exhaustion (TTE) and with improvements in maximal cardiac output ($\dot{Q}$max, from 18.1 ± 1.1 to 20.1 ± 1.2 l/min; $P < 0.01$). Only HIIT produced a significant increase in skeletal muscle mitochondrial oxidative capacities (*V*max) which increased 36.4% after the HIIT. Both training styles resulted in increased capillary density, with a two-fold higher enhancement after continuous training (40%) than for the HIIT (21%). The gain of *V*max was correlated with the gain of time to exhaustion and the gain of $\dot{V}O_{2MAX}$ with HIIT. The gain of $\dot{Q}$max was also correlated with the gain of $\dot{V}O_{2MAX}$. The results of this study reveal that endurance training programs with similar exercise duration and similar total mechanical workload but different O_2 fluctuations, induce specific peripheral and central adaptations. In particular, repeated fluctuations of O_2 consumption during training sessions seem to be necessary to improve muscular oxidative capacities. Together, these results provide a mechanistic framework to explain the greater efficiency of interval over continuous training on endurance performance enhancement. Moreover, our observations suggest that enhancements of muscular mitochondrial function are actively involved in the observed $\dot{V}O_{2MAX}$ improvements. Therefore, HIIT seems optimal in maximizing both peripheral muscle and central cardiorespiratory adaptations, permitting significant functional improvement.

Mechanisms of High Intensity Interval Training

Talanian, Galloway, Heigenhauser, Bonen, Spriet (2007)

Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women

The aim of this study was to examine the effects of seven HIIT sessions over 2 weeks on skeletal muscle substrate content, mitochondrial enzyme activity, fatty acid transport proteins, $\dot{V}O_{2PEAK}$, and whole body metabolic, hormonal, and cardiovascular responses to exercise. Eight female participants performed a $\dot{V}O_{2PEAK}$ test and a 60-min cycling trial at approximately 60% $\dot{V}O_{2PEAK}$ before and after training. Each HIIT session consisted of ten 4-minute bouts at approximately 90% $\dot{V}O_{2PEAK}$ with 2 minutes of rest between intervals. Training increased $\dot{V}O_{2PEAK}$ by 13%. After HIIT, plasma epinephrine and heart rate decreased during the final 30 minutes of the 60-minute cycling trial at approximately 60% of pre-training $\dot{V}O_{2PEAK}$. Exercise whole body fat oxidation increased by 36%, and net glycogen use was reduced during the post-training 60-minute cycling trial. HIIT significantly increased muscle mitochondrial β-hydroxyacyl-CoA dehydrogenase and citrate synthase (31.8% and 19.88%, respectively) after training. In addition, total muscle plasma membrane fatty acid-binding protein content increased significantly (25%), whereas fatty acid translocase/CD36 content was unaffected after HIIT. In summary, seven sessions of HIIT over 2 weeks at a training intensity of approximately 90% $\dot{V}O_{2PEAK}$ was sufficient to elicit significant improvements in $\dot{V}O_{2PEAK}$ and resulted in increases in whole body and skeletal muscle capacity for fatty acid oxidation during exercise in moderately active women.

Perry, Heigenhauser, Bonen, Spriet (2008)

High-intensity aerobic interval training increases fat and carbohydrate metabolic capacities in human skeletal muscle

The purpose of this study was to investigate the effects of 6 weeks of high intensity interval training (HIIT) on the capacity for skeletal muscle and whole-body carbohydrate and fat oxidation in recreationally active young men and women. Eight subjects participated and were asked to complete a $\dot{V}O_{2PEAK}$ test, a test to exhaustion (TE) at 90% of $\dot{V}O_{2PEAK}$, and a 1 hour cycle test at 60% of $\dot{V}O_{2PEAK}$ prior to and after completing and six weeks of cycle ergometer based HIIT that consisted of 10 intervals at 90% $\dot{V}O_{2PEAK}$ for 4 minutes interspersed with 2 minute rest periods. Intensity was maintained by monitoring heart rate and increasing power output as subjects adapted. Relative to performance, improvements were seen in: $\dot{V}O_{2PEAK}$ and training power output. In addition, muscle and blood analyses were performed at PRE and POST testing both at rest and following the TE test. In general terms, these analyses included: muscle enzyme activities, muscle metabolites and glycogen content, and blood measurements. Specifically, improvements were seen in increased skeletal muscle mitochondrial enzyme activity and content, increased transport proteins for fatty acids, glucose, and lactate, and increased resting glycogen. These adaptations were associated with decreased reliance on anaerobic metabolism and greater time to exhaustion at 90% of pre-training $\dot{V}O_{2PEAK}$, as well as increased fat oxidation at 60% pre-training $\dot{V}O_{2PEAK}$. Together, these results indicate that HIIT elicits changes in a large number of physiological variables and likely leads to improved performance through various pathways simultaneously.

Studies Investigating High Intensity Interval Training with Nutritional Interventions

Smith, Walter, Graef, Kendall, Moon, Lockwood, Fukuda, Beck, Cramer, Stout (2009)

Effects of β-alanine supplementation and high-intensity interval training on endurance performance and body composition in men; a double-blind trial

The purpose of this study was to examine the effects of HIIT with either β-alanine supplementation or a placebo on endurance performance and aerobic metabolism in recreationally active college-aged men. Forty-six men were tested for $\dot{V}O_{2PEAK}$, time to fatigue, ventilatory threshold, and total work done at 110% of pre-training $\dot{V}O_{2PEAK}$. In a double-blind fashion, all subjects were randomly assigned into one either a placebo or β-alanine group and engaged in a total of six weeks of HIIT consisting of 5–6 bouts of two-minute intervals with one-minute rest periods. Training followed a fractal periodized plan, which began at an intensity of 90% of peak power achieved during the pretesting $\dot{V}O_{2PEAK}$ test and progressed in an undulating manner, reaching a maximum of 115% of peak power. Significant improvements in $\dot{V}O_{2PEAK}$, time to exhaustion, and total work done after three weeks of training were observed with no significant differences between the groups. The findings of this study which are relevant to the current investigation, are that HIIT significantly increased time to exhaustion and is an effective tool that stimulates significant aerobic improvements with a relatively short period of training.

Jourkesh, Ahmaidi, Keikha, Sadri, Ojagi (2011)

Effects of six weeks sodium bicarbonate supplementation and high-intensity interval training on endurance performance and body composition

The primary aim of this study was to examine the effects of HIIT with or without sodium bicarbonate supplementation on endurance performance and aerobic metabolism. Thirty-six recreationally active college aged men gave their informed consent and volunteer to participate in the study. At baseline, $\dot{V}O_{2PEAK}$, time to fatigue, ventilatory threshold, and total work done at 110% of pre-training $\dot{V}O_{2PEAK}$ were assessed. In a double-blind fashion, subjects were randomly assigned into either a placebo or sodium bicarbonate group. All subjects supplemented four times per day (total of 200 mg/day) for the first 21-days, followed by two times per day (100 mg/day) for the subsequent 21 days, and engaged in a total of six weeks of HIIT consisting of 5-6 bouts of a 2:1 minute cycling work-to-rest ratio. The authors reported significant improvements in $\dot{V}O_{2PEAK}$, time to exhaustion, and total work after three weeks of training. These findings highlight the use of HIIT to induce significant aerobic improvements is effective and efficient.

<u>Metabolism and Mechanisms of β-Hydroxy-β-Methylbutyrate</u>

Nissen, Sharp, Paton, Vukovich, Trappe, Fuller Jr. (2000)

β-Hydroxy-β-Methylbutyrate (HMB) Supplementation in Humans Is Safe and May Decrease Cardiovascular Risk Factors

The purpose of this study was to present the collective safety data from nine studies in which humans were fed 3 grams of HMB per day. The studies ranged from 3 to 8 weeks in duration and the populations studied included males and females, young

and old individuals, and exercising or non-exercising populations. Organ and tissue function was assessed by blood chemistry and hematology; in addition, mild effects on emotional perception were assessed using an emotional profile test (Circumplex) and tolerance of HMB was assessed with a battery of 32 health-related questions. HMB did not adversely affect any surrogate marker of tissue health and function. The emotion profile indicated that HMB significantly decreased one indicator of negative mood, reflecting a more positive mindset. No negative effects of HMB were indicated. Compared with the placebo, HMB supplementation resulted in a significant decrease in total cholesterol of 5.8%, LDL cholesterol of 7.3% and systolic blood pressure (4.4 mm Hg, $P < 0.05$). These effects of HMB on surrogate markers of cardiovascular health could result in a decrease in the risk of heart attack and stroke. The only definitive effects of HMB were positive in nature, especially relating to lowering plasma cholesterol and blood pressure. In conclusion, the objective data collected across nine experiments indicate that objective measures of health and perception of well-being are generally enhanced after HMB consumption without any apparent negative effects. Therefore, the authors advise that HMB can be taken safely as an ergogenic aid for exercise and that the use of supplementing 3 grams per day of HMB as an ergogenic aid for exercise and it is well tolerated and safe in humans.

Holecek, Muthny, Kovarik, Sispera (2009)

Effect of β-hydroxy-β-methylbutyrate (HMB) on protein metabolism in whole body and in selected tissues

The aim of this study was to examine the effect of HMB administration on leucine and protein metabolism in whole body and to estimate changes in protein synthesis and proteolysis in selected tissues. Two tracers ($_{L}$-[1-^{14}C] leucine and $_{L}$-[3,4,5-^{3}H] phenylalanine) were used to test the possible effect of interference of HMB and leucine on metabolism and to avoid its effect on interpretation of the obtained results. Male Wistar rats had a cannula inserted into the jugular vein and a dose of 0.1 g/kg of body weight HMB was administered via the cannula for ½ of the dose and the other ½ of the dose was given subcutaneously. The control group for this study received saline administered in the same method. Whole-body protein metabolism were evaluated 24 h later using $_{L}$-[1-^{14}C] leucine and $_{L}$-[3,4,5-^{3}H] phenylalanine. Changes in proteasome dependent proteolysis and protein synthesis were determined according to the ‘‘chymotrypsin-like” enzyme activity as determined using the fluorogenic substrate Suc LLVY-MCA and labeled leucine and phenylalanine incorporation into the protein. Amino acid concentrations in de-proteinized samples of blood plasma or tissues were determined with HPLC. A decrease in leucine clearance (control =1053 ± 36, HMB = 870 ± 52; $p<0.05$) and whole-body protein turnover interpreted as a decrease in whole-body proteolysis (control =172 ± 9, HMB = 144 ± 8; $p<0.05$) and protein synthesis (control =107 ± 8, HMB = 84 ± 5; $p<0.05$) was discovered in HMB treated rats. Proteasome-dependent proteolysis decreased significantly in skeletal muscle alone (control =6.9 ± 0.9, HMB = 3.8 ± 0.3; $p<0.05$), while changes in heart, liver, jejunum,

colon, kidney, and spleen were non-significant. Decreases in protein synthesis were observed in the heart, colon, kidney, and spleen, while an increase was observed in the liver. No significant changes in leucine oxidation were observed. The main effect of HMB administration on protein metabolism seems to be the inhibition of proteasome dependent proteolysis in skeletal muscle due to decreased levels of glutamate, glutamine and alanine in the blood. The data also indicate that HMB is partly responsible for the inhibitory effect of exogenous leucine on proteolysis and not for its stimulatory effect on protein synthesis. The authors conclude that the protein anabolic effect of HMB in skeletal muscle is related to inhibition of proteolysis in proteasome. Alterations in protein synthesis in visceral tissues may affect several important functions and the metabolic status of the whole body.

Bruckbauer, Zemel, Thorpe, Akula, Stuckey, Osborne, Martin, Kennel, Wall (2012)

Synergistic effects of leucine and resveratrol on insulin sensitivity and fat metabolism in adipocytes and mice

The authors of this study sought to determine whether leucine would exhibit synergy with low levels of resveratrol on sirtuin-dependent outcomes in adipocytes and in diet-induced obese mice. One of the primary goals of the research was to investigate whether leucine and/or HMB interact with resveratrol, a plant polyphenol found in the skin of red grapes and in other fruits to act as another Sirt1 activator, in Silent Information Regulator Transcript 1(Sirt1) activation and downstream effects. Sirt1 and Sirt3 stimulation leads to activation of mitochondrial biogenesis and metabolism, resulting in increased fatty acid oxidation. To accomplish this, two studies were

performed, one in cell culture and then a second experiment extended to an in vivo mouse study where the downstream effects of Sirt1 activation were also measured. 3T3-L1 mouse pre-adipocyte cells were treated with leucine (0.5 mM), HMB (5 μM) or resveratrol (200 nM) alone or in combination. In addition, diet-induced obese mice were treated for 6-weeks with low (2 g/kg diet) or high (10 g/kg diet) dose HMB, leucine (24 g/kg diet; 200% of normal level) or low (12.5 mg/kg diet) or high (225 mg/kg diet) dose resveratrol, alone or as a combination with leucine-resveratrol or HMB-resveratrol. The combinations leucine-resveratrol or HMB-resveratrol compared to the individual treatments significantly increased fatty acid oxidation, AMPK, Sirt1 and Sirt3 activity in 3T3-L1 adipocytes and in muscle cells. Similarly, 6-week feeding of low-dose resveratrol combined with either leucine or its metabolite HMB to diet-induced obese mice increased adipose Sirt1 activity, muscle glucose and palmitate uptake, insulin sensitivity, improved inflammatory stress biomarkers (CRP, IL-6, MCP-1, adiponectin) and reduced adiposity. The data from this study demonstrates that either leucine or its metabolite HMB may be combined with a low concentration of resveratrol to exert synergistic effects on Sirt1-dependent outcomes; this may result in more practical dosing of resveratrol in the management of obesity, insulin-resistance and diabetes. Additionally, diet-induced obese mice supplementing on HMB alone resulted in a significant change in insulin sensitivity and muscle glucose uptake. Another novel finding of this study was that HMB, whether combined with resveratrol or alone, resulted in a significant increase in AMPK activation and on the β–activation in the adipocytes.

Wilkinson, Hossain, Hill, Phillips, Crossland, Williams, Loughna, Churchward-Venne, Breen, Phillips, Etheridge, Rathmacher, Smith, Szewczyk, Atherton (2013)

Effects of leucine and its metabolite β-hydroxy-β-methylbutyrate on human skeletal muscle protein metabolism

This study investigated the possibility that HMB could represent an anabolic metabolite of leucine by studying the effects of HMB_{FA} on human muscle protein turnover and compared with that of leucine. The authors carried the following three hypotheses into the experiment: 1) HMB_{FA} provision would acutely stimulate MPS; 2) HMB_{FA} would stimulate muscle protein synthesis through mechanisms similar to its precursor, leucine; 3) HMB_{FA} would also acutely depress muscle protein breakdown. Eight healthy young men, who were recreationally active but not involved in a formal training program, were recruited for the study. On the morning of the study, subjects had an 18 g cannula inserted into the antecubital vein of one arm for tracer infusion, a retrograde 14 g cannula inserted to sample arterialized blood from the dorsal capillary bed of the hand and – in the HMB trial only – had blood-sampling catheters inserted into the common femoral vein. The researchers chose not to place the added burden of femoral lines on the subjects in the leucine study due to the confounding factor of insulin secretion associated with leucine, since studies using large doses of AAs have failed to show an effect on muscle protein breakdown when insulin is clamped. A primed, continuous infusion of [1,2-^{13} C2] leucine tracer– and in the HMB trial [$^{2}H_5$] phenylalanine– was started (at $t = -2.5$ hr) after the first biopsy and maintained until the end of the study (+2.5 hr). During the first 2.5 hr period baseline measurements were gathered. The participants then ingested either 3.42 g of a buffered and flavored HMB_{FA}

solution (which provided 2.42 g of HMB), or 3.42 g of L-leucine along with ~400 ml of water. Muscle biopsies (~200 mg) were taken from the vastus lateralis, under sterile conditions using a local anesthetic. Post-absorptive plasma glucose concentrations were measured using an ILab 300 Plus Chemistry Analyser and plasma insulin concentrations were measured using undiluted samples on a high-sensitivity ELISA. Amino acid concentrations were analyzed on a dedicated amino acid analyzer utilizing a lithium buffer separation. Plasma HMB and amino acid concentrations were analyzed by gas chromatography–mass spectrometry. Sarcoplasmic protein concentrations for determination of muscle protein synthesis were evaluated via immunoblotting. Muscle protein breakdown was calculated via arteriovenous dilution of the [$^2H_5$]-phenylalanine tracer. The results of the study indicated that orally consumed free-acid HMB exhibited rapid bioavailability in plasma and muscle and stimulated muscle protein synthesis—increase of 70%, similarly to 3.42 g leucine which increased muscle protein synthesis by 110%. While HMB_{FA} and leucine both increased anabolic signaling via the mTOR pathway, this was more pronounced with leucine. The phosphorylation of p70S6K1 increased similarly in both HMB_{FA} (~56%) and leucine (~45%) groups at 30 min. However, by the 90 min time point increased p70S6K1 phosphorylation was maintained (~71%) only in the leucine group. HMB_{FA} consumption also attenuated muscle protein breakdown, decreasing breakdown by 57% in an insulin-independent manner. The authors conclude that oral consumption of HMB in free-acid form rapidly elevated plasma and intramuscular HMB bioavailability from fasting concentrations and that

exogenous HMB_{FA} induces acute muscle anabolism via increased muscle protein synthesis and reduced muscle protein breakdown although there is still uncertainty if HMB_{FA} acts separately or in conjunction with the mechanism(s) of leucine.

Pinheiro, Gerlinger-Romero, es-Ferreira, de Souza-Jr, Vitzel, Nachbar, Nunes, Curi (2012)

Metabolic and functional effects of β-hydroxy-β-methylbutyrate (HMB) supplementation in skeletal muscle

The aim of this study was to evaluate the effects of HMB_{Ca} supplementation for 4 weeks on metabolic parameters and skeletal muscle contractile function in rats. The authors measured the ATP and glycogen content, citrate synthase activity, maximum strength production, resistance to acute fatigue, contraction velocity and relaxation capacity in skeletal muscle. Wistar rats were supplemented with 320 mg/kg of body weight per day HMB_{Ca} for 4 weeks. A placebo group received the same volume of a saline solution. The animals were anesthetized and both hind limbs were fixed on an acrylic platform. Direct electrical stimulation of the sciatic nerve was used to assess both tetanic force and muscle twitch strength and resistance to acute muscle fatigue of the gastrocnemius muscle. The gastrocnemius muscle was dissected into red and white portions, which were evaluated for ATP and glycogen content via commercially available kits. The effect of HMB_{Ca} on citrate synthase activity in the white and red portions of gastrocnemius muscle was determined by spectrophotometer. No change in gastrocnemius muscle mass was observed in HMB_{Ca}-supplemented rats compared to placebo group. Muscle tetanic force was increased by HMB_{Ca} supplementation;

however, no change was observed in time to peak of contraction and relaxation time. Resistance to acute muscle fatigue during intense contractile activity was also improved after HMB_{Ca} supplementation and glycogen content was increased fivefold in white and fourfold in red portions of gastrocnemius muscle. HMB_{Ca} supplementation also doubled the ATP content in red muscle cells and increased ATP concentrations in white portions of gastrocnemius muscle by 1.2-fold. Citrate synthase activity was increased by twofold in red portion of gastrocnemius muscle. These results support the premise that HMB supplementation results in metabolic changes that are associated with increased muscle strength generation and prevention of acute muscle fatigue via a marked change in oxidative metabolism during intense contractions.

Fuller, Sharp, Angus, Baier, Rathmacher (2011)

Free acid gel form of β-hydroxy-β-methylbutyrate (HMB) improves HMB clearance from plasma in human subjects compared with the calcium HMB salt

The present study was designed to examine whether HMB in free acid gel form (HMB_{FA}) could improve bioavailability to tissues over the previous calcium salt form of HMB (HMB_{Ca}). To study this, the researchers designed two longitudinal cross-over studies. In each study four males and four females were given three treatments: 1) 1g HMB_{Ca} 2) equivalent HMB_{FA} swallowed and 3) HMB_{FA} held sublingual for 15 s then swallowed. For study 1, blood samples were obtained at 2, 5, 10, 15, 25, 35, 45, 60, 90, 120 and 180 min after ingestion. In study 2, additional blood samples were also obtained at 360, 720 and 1440 min after supplementation. Plasma and urine HMB were analyzed by gas chromatography–mass spectrometry, while portions of the pre-ingestion and 180

min blood samples (study 1) and samples from 1440 min (study 2) were used for measurements of glucose, uric acid, blood urea nitrogen, creatinine, Na, K, Cl, CO_2, P, protein, albumin, globulin, albumin:globulin ratio, total bilirubin, direct bilirubin, alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, g-glutamyl transpeptidase, Fe-binding capacity, unsaturated Fe-binding capacity, Fe, Fe saturation, total cholesterol, TAG, HDL, LDL and cholesterol ratio were sent to a commercial lab for analysis. Treatment with HMB did not result in differences in any of the measured parameters for blood chemistry. Plasma HMB was measured for 3 h following treatment in study 1 and 24 h with urine collection in study 2. In both the studies, the time to peak plasma HMB were significantly different between the forms of HMB, with 128min for the HMB_{Ca}, 38 min for the HMB_{FA} swallowed immediately and 38 min for the HMB_{FA} held sublingually. Similarly, the authors also a found significant difference in the peak concentrations between the different forms of HMB: 131 μmol/l for the HMB_{Ca}, 249 μmol/l for the HMB_{FA} swallowed and 239 μmol/l for the HMB_{FA} held sublingually. In addition, retention of HMB was significantly higher in free acid form of HMB versus the calcium salt form as the daily urinary HMB excretion was not significantly increased despite increases in plasma concentration. Finally, the plasma half-life of HMB was significantly different between the treatments of the two forms of HMB: 3.17 hr for the HMB_{Ca}, 2.50 hr for HMB_{FA} swallowed and 2.51 hr for HMB_{FA} held sublingually. In summary, HMB delivery by free acid gel results in a faster and greater peak in HMB plasma concentration as well as equally sustained concentration compared with the HMB_{Ca} administered in a capsule, is equally safe and may improve HMB availability and efficacy to tissues in health and disease.

Effect of β-Hydroxy-β-Methylbutyric Free Acid on Recovery

Wilson, Lowery, Joy, Walters, Baier, Fuller Jr, Stout, Norton, Sikorski, Wilson, Duncan, Zanchi, Rathmacher (2013)

β-Hydroxy-β-Methylbutyrate free acid reduces markers of exercise-induced muscle damage and improves recovery in resistance-trained men

This study was designed to determine the effects of short-term with HMB_{FA} supplementation administered 30 minutes before a bout of resistance exercise on markers of muscle damage, protein breakdown, recovery, and hormone status in resistance-trained athletes. Twenty resistance-trained males were recruited to participate in a high-volume resistance training session comprised of three sets of twelve maximal repetitions intensity in each exercise, with a supervised and timed rest period of 1 min between the sets. The exercises included in the workout were: full squats, bench press, dead lifts, pull-ups, barbell bent over rows, parallel dips, military press, barbell curls and triceps extensions. Participants were randomly assigned to receive either 3 g/d of HMB_{FA} or a placebo divided equally in three servings and to be consumed: 30 min before exercise, with lunch, and with an evening meal. Resting blood draws to evaluate serum creatine kinase, urinary 3-methylhistadine, testosterone, and cortisol were obtained immediately before the exercise session and 48 h post-exercise. Serum total testosterone, cortisol and C-reactive protein were assessed using ELISA kits. Serum creatine kinase (CK) was measured using colorimetric procedures at 340 nm. The results showed that CK increased 329% in the placebo group, while the HMB_{FA} group saw only a 104% increase above baseline. Participants also reported a significant change for their perceived recovery status, in which responses decreased to a greater extent in the placebo— 9.1

immediately prior to exercise and 4.6 48 hr post exercise—than in the HMB_{FA} group—9.1 immediately prior to exercise to 6.3 48 hr post exercise— meaning the placebo group felt less recovered and more likely to do poorly is subsequent exercise. Muscle protein breakdown, measured by 3-methylhistadine analysis, decreased 3.94% with HMB_{FA} supplementation and approached significance while no change was observed in the placebo group. There were no reported changes in plasma total or free testosterone, cortisol or C-reactive protein. In conclusion, these results suggest that an HMB_{FA} supplement given to trained individuals prior to intense exercise can attenuate increases in markers of muscle damage and may improve perceived readiness for subsequent trainings following a high-volume, muscle-damaging resistance-training session.

Effects of Endurance Training and β-Hydroxy-β-Methylbutyrate Supplementation

Park, Henning, Grant, Lee, Lee, Arjmandi, Kim (2013)

HMB attenuates muscle loss during sustained energy deficit induced by calorie restriction and endurance exercise

The purpose of this study was to investigate the efficacy and underlying mechanisms of HMB on lean body mass, muscle mass and physical performance under normal training conditions with ad libitum diet versus catabolic conditions induced by prolonged endurance exercise combined with caloric restriction in male mice. The authors' hypotheses were that supplementation with HMB_{Ca} would enhance muscle mass and physical performance under normal training conditions and would help to attenuate the loss of muscle mass and physical performance under catabolic conditions. For this study, 61 six-week old C57BL/6 male mice were divided into three baseline groups: 1)

TB = true baseline, sedentary control (n = 7); 2) B = baseline (n = 27); and 3) BH = baseline + HMB_{Ca} (0.5 g/kg BW/d) (n = 27). Groups B and BH underwent a four-week run-in phase to simulate initial entry training that soldiers go through upon entering the military where mice exercised three days a week for one hour each day at a speed of 6 m/min (i.e. fast walk) on a forced exercise wheel. After this initial period, 7 mice from each group were removed from the study and utilized for baseline measures. The 20 remaining mice in the B group were then randomly assigned into ALT [= ad libitum-trained (exercised 1 h/d for 3 d/wk, 6 m/min)] and C [= caloric restricted (−30% of ad libitum groups) + trained (~6 h/d = 2 km/d, 6 d/wk, 6 m/min speed)] groups. The 20 remaining mice from the BH group were randomly assigned into ALTH [= ad libitum-trained + HMB_{Ca} (0.5 g/kg BW/d)] and CH (= C + HMB_{Ca} groups) (n = 10/group). The second portion of the training experiment lasted 6 weeks. Repeated in vivo assessments included body composition via DEXA, grip strength and sensorimotor coordination and were performed after the initial 4 week initiation portion and after the 6 week experimental protocol. In vitro analyses included muscle wet weights, expression of selected genes and proteins regulating muscle mass, and myofiber cross-sectional area. There was no significant difference pre to post between the ALT and ALTH groups for total body mass. Both of the groups that were fed an ad libitum diet increased in weight over the course of the 6 week training period. However, the ALTH group had 17% greater lean body mass than ALT after the training period. Conversely, in the catabolic groups, the group without HMB_{Ca}, C group, had 17% greater lean body mass than the HMB_{Ca} group, CH, after the experimental protocol. Fat mass increased 25% in ALT, this created a 12%, significant difference between the ALT and ALTH groups after the

training protocol. Both catabolic groups had significantly lower fat mass than the normal training groups after the training protocol. Interestingly, fat mass decreased by 56% in the C group, but only 38% in the CH after the training period. The only group where a decrease in grip strength occurred was group C, where a 10% decrease was observed. Grip strength was maintained in CH and following the protocol, group CH had an 11% greater grip strength than the C group. Gastrocnemius mass was significantly greater (+10%) in CH than C following catabolic conditions. Similarly, the mean cross-sectional area of C was 35% lower compared to CH after the experimental protocol. Therefore, the group supplementing with HMB_{Ca}, CH, significantly attenuated the decrease in fiber cross-sectional area of the gastrocnemius. Finally, gastrocnemius atrogin-1 mRNA expression was elevated in C but not in CH compared to all other groups. Atrogin-1 protein levels, however, showed no significant changes. In conclusion, the major findings reported by the authors are that HMB_{Ca} intake during a catabolic condition attenuates loss of strength, muscle mass and myofiber cross-sectional area, but not lean mass as measured via DEXA. It is also relevant that HMB_{Ca} increased lean body mass, attenuated increases in fat mass, and improved sensorimotor function under normal training conditions.

Knitter, Panton, Rathmacher, Petersen, Sharp (2000)

Effects of β-hydroxy-β-methylbutyrate on muscle damage after a prolonged run

This study examined the effects of supplementing HMB_{Ca} on muscle damage as a result of an acute bout of intense continuous endurance exercise. All participants involved in this study regularly partook of endurance training, only subjects running at

least 48 km/week were selected to participate. Participants, 8 males and 8 females, were paired according to their 2-mile run times and past running experience. Each pair was randomly assigned a treatment of either HMB_{Ca} (3 g/day) or a placebo. Participants supplemented three times a day at meal times for 6 weeks prior to the run, and for 4 days after the run. Daily training was allowed to occur ad libitum. All participants then took part in an acute bout of continuous endurance exercise in the form of a 20 km run. Blood samples were taken prior to supplementation, 4 weeks after supplementation began, immediately after completion of the prolonged run, and each day for 4 days following the prolonged run. Serum samples were analyzed for creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) activity by a commercial laboratory. Plasma was collected and analyzed for HMB by gas chromatography-mass spectrometry. The placebo group exhibited a significantly greater increase in CPK activity in the 4-day period after prolonged run than did the HMB_{Ca} group (Day 1 = 37%, Day 2 = 39%, Day 3=38.5%, Day 4=11.3%). Additionally, when covaried for pre-run values, LDH activity was significantly lower in the HMB_{Ca} group compared with the placebo group. In conclusion, the authors suggest that the fact that the placebo group exhibited higher LDH activity after the prolonged run compared with the HMB_{Ca} group suggests that they would tend to sustain more muscle damage as a result of the run. This fact combined with the lower CPK levels of the HMB_{Ca} group support the authors' hypothesis that HMB_{Ca} supplementation may help attenuate muscle damage following high intensity exercise.

Vukovich, Dreifort (2001)

Effect of β-Hydroxy β-Methylbutyrate on the Onset of Blood Lactate Accumulation and $\dot{V}O_{2PEAK}$ in Endurance-Trained Cyclists

The purposes of this study were to: 1) investigate the effects of HMB_{Ca} supplementation on the onset of blood lactate accumulation and $\dot{V}O_{2PEAK}$ and in endurance-trained cyclists, both indicators of training status; 2) examine the effect of acute exercise on plasma HMB concentrations. Eight male master-level competitive cyclists participated in the study. Training volume of the subjects was ~280–330 miles per week. The training was allowed to proceed ad libitum and consisted of intervals (aerobic and anaerobic), sprints, and racing. Participants were randomly assigned to complete three 2-week supplementation periods (HMB_{Ca}, leucine, or placebo) each followed by a 2-week washout period. The study utilized a crossover design, so that subjects acted as their own controls. For the $\dot{V}O_{2PEAK}$ and time to reach $\dot{V}O_{2PEAK}$ test participants cycled at a rate of 90 rpm at 150 W. Wattage was increased by 25 W every 3 minutes until the subject could no longer maintain 80 rpm. The fractional concentrations of oxygen and carbon dioxide in the expired air were analyzed with a Quinton Q-Plex 1 metabolic cart. Researchers collected blood samples during the last 20 seconds of each stage from a catheter inserted into a forearm vein for determination of blood lactate, plasma glucose, HMB, and free fatty acids. Immediately after the completion of the test a blood sample was obtained for the determination of blood lactate concentration associated with $\dot{V}O_{2PEAK}$. The supplementation protocol consisted of 3 g of cornstarch$\cdot day^{-1}$ (CON), 3 g of $HMB_{Ca}\bullet day^{-1}$, and 3 g of leucine$\bullet day^{-1}$ (LEU). Capsules were identical in size and appearance and participants were instructed to consume 4

capsules 3 times per day for a total of 12 capsules per day. A 1-way RMANOVA on relative changes in $\dot{V}O_{2PEAK}$ and time to reach $\dot{V}O_{2PEAK}$ resulted in a significant increase of 4% in $\dot{V}O_{2PEAK}$ and 3.5% in time to reach $\dot{V}O_{2PEAK}$ during the HMB trial, which was greater than the leucine or placebo trials. Two-way RMANOVA resulted in a significant time and treatment effect. The onset of blood lactate accumulation was significantly higher after HMB (9.1% increase pre to post) supplementation compared with both the placebo (0.75%) and leucine (2.1%) trials. The relative increase in $\dot{V}O_2$ at 2 mM blood lactate, as analyzed by a one-way RMANOVA, was also significantly greater after HMB (8.6% increase pre to post) supplementation compared with leucine (4.2%) or placebo (2.1%) trials. Although the results of the current study lend support the hypothesis that HMB_{Ca} supplementation may have positive effects on aerobic performance by delaying the onset of blood lactate accumulation, the authors caution that they believe no adaptations occurred. Vukovich and Dreifort do offer up a further hypothesis that if HMB_{Ca} prevents protein breakdown and increases protein synthesis, then cellular and mitochondrial proteins may increase. This may result in improvements in the oxidative system in which electrons from NADH, formed within the cytosol, are shuttled into the mitochondria allowing for increased oxidative phosphorylation, rather than producing lactate through the reduction of pyruvate.

Lamboley, Royer, Dionne (2007)

Effects of β-hydroxy-β-methylbutyrate on aerobic-performance components and body composition in college students

The aim of this study was to determine the effects of the HMB_{Ca} supplementation coupled with a 5-week interval-training program, in active college students unaccustomed to this kind of training, on selected components of aerobic performance and body composition. Eight men and eight women were randomly assigned to either an HMB_{Ca} or a placebo group for a 5-wk supplementation period during which they underwent interval training on a treadmill 3 times per week. Body composition, including measurements of total body mass, as well as body fat and lean body mass, was determined using DEXA. An incremental continuous test to exhaustion on a treadmill was performed using a breath-by-breath gas analyzer. Researchers collected respiratory gases to measure $\dot{V}O_{2MAX}$, as well as ventilatory threshold (VT) and respiratory compensation point (RCP). VT was determined to be the intensity corresponding to an increase in $\dot{V}_E$:$\dot{V}O_2$ without a simultaneous increase of $\dot{V}_E$:$\dot{V}CO_2$, the first sustained rise in excess CO_2, and the first increase in the slope of $\dot{V}CO_2$ versus $\dot{V}O_2$. The authors measured the RCP as the intensity corresponding to an increase in both $\dot{V}_E$:$\dot{V}O_2$ and $\dot{V}_E$:$\dot{V}CO_2$, the second sustained rise in excess CO_2, and the second increase in the slope of $\dot{V}CO_2$ versus $\dot{V}O_2$. Participants were next tested to measure the time to exhaustion at the maximal aerobic speed—defined as the lowest speed that elicited $\dot{V}O_{2MAX}$ during the graded exercise test. An interval training cycle consisted of 5 exercise bouts with each bout equal to 100% of an individual's total time as measured by the time to exhaustion test. Therefore, for each workout, an individual began with a warm-up period of 5 min at

50% of his or her maximal aerobic speed. After this warm-up, an initial period of active recovery running began at an intensity equivalent to 60% of maximal aerobic speed followed immediately by a fast run at an intensity equivalent to 100% of maximal aerobic speed. Therefore, the slow run and fast run of each interval training cycle were each of the same duration: 50% of the total time to exhaustion. $\dot{V}O_{2MAX}$ for both groups significantly increased after the exercise intervention. However, when the investigators expressed this as a percentage of increase, it indicated that $\dot{V}O_{2MAX}$ increased significantly more in the HMB_{Ca} group (15.5%) than in the placebo group (8.4%). The researchers found no significant difference in any measure of body-composition data between the pre- and posttest for either studied group. Furthermore, they discovered no significant difference between the 2 groups for all body-composition variables after the training and supplementation period. Although the time to exhaustion decreased significantly in both groups, the authors found that again, the HMB_{Ca} supplemented group (-42.4%) was significantly different from the placebo group (-27.1%). Further, the researchers discovered that ventilatory threshold was improved significantly in both groups, with no significant difference between the two supplemental interventions. Similarly, there was a significant increase in respiratory compensation point in both interventions. However, as with the $\dot{V}O_{2MAX}$, when expressed as a percentage of $\dot{V}O_{2MAX}$, respiratory compensation point improved significantly more for the HMB group (13.4%) than for the PLA group (8.4%). The authors concluded that although the mechanisms whereby HMB influences some components of sport performance are unknown, supplementation with HMB coupled with a high-intensity interval training program induced a greater increase in $\dot{V}O_{2MAX}$ and in RCP in active subjects, thereby

positively affecting selected components of aerobic performance in active college students.

Robinson, Stout, Miramonti, Fukuda, Wang, Townsend, Mangine, Fragala, Hoffman (2014)

High-intensity interval training and β-hydroxy-β-methylbutyric free acid improves aerobic power and metabolic thresholds

The purpose of this investigation was to examine the effects of cycle ergometer based HIIT and supplementation with 3 grams per day of HMB_{FA} on maximal oxygen consumption ($\dot{V}O_{2PEAK}$), ventilatory threshold (VT), respiratory compensation point (RCP), time to exhaustion (T_{MAX}), and peak power (P_{PEAK}) in college-aged men and women. Thirty-four recreationally active men and women participated in the study and were randomly assigned to either control or one of the HIIT groups: placebo (PLA-HIIT) or HMB_{FA} supplemented (HMB_{FA}-HIIT). At baseline, participants completed a graded exercise test to determine $\dot{V}O_{2PEAK}$, VT, RCP, and T_{MAX}. Subsequently, PLA-HIIT and HMB_{FA}-HIIT completed 12 HIIT sessions consisting of 5-6 intervals at a 2:1 work to rest ratio with intensities ranging from 85-120% of P_{PEAK} over the course of 4 weeks while supplementing with either a placebo or 3 g/day of HMB_{FA}, while the control group was asked to maintain normal diet and exercise patterns. The authors reported that both PLA-HIIT and HMB_{FA}-HIIT groups resulted in significant increases in T_{MAX} and RCP compared to control, but HMB_{FA}-HIIT saw a significant increase in $\dot{V}O_{2PEAK}$ and VT compared to control and PLA-HIIT, despite not significant differences in training volumes between HIIT groups. These results support the hypothesis that HMB_{FA} may

augment at least some of the physiological adaptations associated with HIIT; the authors postulate that these findings may be the result of HMB related increases in mitochondrial biogenesis, fat oxidation, and improvements in metabolic processes, and that these benefits may be mediated through the regulation of adenosine monophosphate kinase and sirtuin activity in muscle cells.

Neuromuscular Fatigue Thresholds

deVries, Tichy, Housh, Smyth, Tichy, Housh (1987)

A method for estimating physical working capacity at the fatigue threshold (PWC_{FT})

The purpose of this study was to evaluate a new method for determining the highest power output that does not elicit increases in EMG amplitude over time. This new protocol was presented as an alternative to the supramaximal EMG_{FT} testing protocol which may not be suitable for all populations. The testing consisted of 2 minute bouts of exercise at a constant power output while EMG signals were recorded. The stages increased in power output until a stage resulted in a significant increase in average RMS over a two minute stage. Test-retest reliability was found to be 0.947 with no significant difference between trials, showing that a submaximal test to determine the physical working capacity at fatigue threshold is a reliable alternative to the EMG_{FT}. In addition, the authors correlated PWC_{FT} with onset of blood lactate accumulation (OBLA), percent of heart rate range (%HRR), heart rate-workload relation (HR-WL), and critical power (CP). PWC_{FT} were correlated at 0.67 with no significant difference between means, demonstrating the objectivity and validity of the PWC_{FT} test.

deVries, Brodowicz, Robertson, Svodoba, Schendel, Tichy, Tichy (1989)

Estimating physical working capacity and training changes in the elderly at the fatigue threshold (PWC_{FT})

The goal of this study was to evaluate the use of the PWC_{FT} test in an elderly population, to determine if it was sensitive to changes following training, and to determine the minimum exercise intensity appropriate for elderly populations. Twenty-seven healthy, older adults participated in the study and completed either a low (n = 10) or high intensity (n = 7) exercise program 3 days per week for 10 weeks, or were asked to maintain their normal daily routines (control, n = 10). All groups completed a PWC_{FT} test prior to and after the training period. There was no change in PWCFT in the control group and a 29.8% improvement in the low intensity exercise group and a 38.4% improvement in the high intensity exercise group, but there was no significant difference between the low and high intensity exercise groups. These results demonstrate the applicability of this test for assessing fitness in an elderly population.

deVries, Housh, Johnson, Evans, Tharp, Housh, Hughes (1990)

Factors affecting the estimation of physical working capacity at the fatigue threshold

The purpose of this study was to investigate the effects potential methodological changes might have on the physical working capacity at fatigue threshold (PWC_{FT}). The modifications studied were: use of a continuous protocol, use of a treadmill in place of a cycle ergometer, use of a bipolar EMG system in place of the previously used unipolar system, and potential for residual fatigue when tests are completed on consecutive days.

There was no significant mean difference between the results of continuous and discontinuous protocols. For treadmill testing the bipolar lead system was utilized to counteract the increased noise associated with the treadmill motor and excess movement. The heart rate at PWC_{FT} from the treadmill and cycle ergometer trials was well correlated (0.833) and there was no significant mean difference. The bipolar arrangement however, resulted in significantly smaller voltages at any given power output on a cycle ergometer when compared to the unipolar arrangement and the PWC_{FT} from the bipolar test was low to moderately correlated (0.60) with the PWC_{FT} from the unipolar test. Lastly, the authors reported a correlation of 0.812 between the first and second trials when completed 24 hours apart, with no significant mean differences between trials, indicating that the PWC_{FT} test does not result in residual fatigue. The authors also concluded that while the continuous, cycle ergometer based PWC_{FT} method was valid and reliable, further research needs to be done to validate the treadmill test.

Stout, Cramer, Mielke, O'Kroy, Torok, Zoeller (2000)

Effects of 28 days of β-alanine and creatine monohydrate on the physical working capacity at neuromuscular fatigue threshold

This study used physical working capacity at neuromuscular fatigue threshold (PWC_{FT}) to examine the effects of 28 days of β-alanine and creatine monohydrate supplementation. Fifty-one men participated in this double-blind placebo controlled study and were randomly assigned to one of four groups: placebo (PLA), creatine monohydrate (CrM, 5.25 g), β-alanine (b-Ala, 1.6 g), or a combination of creatine monohydrate and β-alanine (CrBA, 5.25 g CrM + 1.6 g b-Ala). The study included a 6

day loading phase of 4 doses per day followed by a further 22 days of 2 doses per day. Prior to and following the supplementation protocol participants completed a continuous, incremental cycle ergometry test during which surface EMG signals were collected for later analysis. PWC_{FT} was determined as the average of the highest power output that resulted in a non-significant increase in amplitude with the lowest power output that resulted in a significant increase in amplitude. The authors reported significant increases in PWC_{FT} in the groups supplementing with β-alanine (b-Ala and CrBA) compared to placebo, with no other differences between groups and no additive effect of creatine monohydrate with β-alanine. The authors posit that the improvements in PWC_{FT} were the result of improved buffering capacity with b-Ala supplementation as a result of increased intramuscular carnosine content, thereby delaying the accumulation of metabolites such as lactate, ammonia, and H^+ ions which are associated with increases in EMG amplitude. Therefore, it is suggested that an improved PWC_{FT} reflects an increased ability to buffer H^+ and delay the exercise induced decrease in pH associated with exercise above the anaerobic threshold. It is important to note however, that other physiological changes that increase oxidative capacity would increase the anaerobic threshold and thereby delay the onset of fatigue through mechanisms unrelated to buffering capacity.

Matsumoto, Ito, Moritani (1991)

The relationship between anaerobic threshold and electromyographic fatigue threshold in college women

The primary aim of this study was to examine the relationship between anaerobic threshold (AT) as determined from gas exchange measures and electromyographic fatigue threshold (EMG_{FT}) as determined from electromyography based fatigue curves collected during 1 minute bouts of constant load exercise at fatiguing intensities; secondarily, in the process of examining this relationship the authors validated the EMG_{FT} method used in the study. Twenty female college students with varied training backgrounds participated in the study. First, they completed a graded exercise test to determine $\dot{V}O_{2PEAK}$ and AT. Next, they completed four 1-minute trials at 350, 300, 250, and 200 W, during which EMG amplitude was collected from the vastus lateralis using surface electrodes. The rate of increase in EMG amplitude was calculated for each trial and the slope coefficients were plotted against power output, resulting in a linear relationship. This was then extrapolated to estimate the y-intercept, which in theory represents the highest power output at which one would observe a zero-slope of EMG amplitude during a trial, which in turn should relate to the AT. The y-intercept was considered the EMG_{FT} and the authors found that it was significantly correlated with the AT determined by gas exchange analysis ($r = 0.823$), leading them to conclude that EMG_{FT} may be an attractive alternative for determining AT.

Moritani, Takaishi, Matsumoto (1993)

Determination of maximal power output at neuromuscular fatigue threshold

The purpose of this investigation was to determine the maximal power output at the neuromuscular fatigue threshold (EMG_{FT}). Twenty male and female college students were recruited to participate in this study. Participants completed a graded exercise test using respiratory gas collection to determine anaerobic threshold, defined as the departure from linearity in the relationship between $\dot{V}_E$ and $\dot{V}CO_2$. They also completed two EMG_{FT} trials: a familiarization and a baseline test, during which EMG data was collected from the vastus lateralis. For each trial, participants completed four 1-minute bouts of exercise at four different power outputs which were selected to be fatiguing intensities. EMG_{FT} values were determined by finding the rate of increase of the amplitude across each bout (slope), and plotting the slope coefficients against power output in order to estimate the y-intercept of a regression line derived from the slope coefficients. The y-intercept of this line represents the power output which would, in theory, result in no increase in EMG across an exercise bout, and therefore should reflect the AT. The authors reported that the EMG_{FT} was highly correlated with AT ($r = 0.92$). Of note, however, is finding that the $\dot{V}O_2$ at EMG_{FT} was significantly higher than the $\dot{V}O_2$ at AT; as a result, the authors speculated that EMG_{FT} may in fact be more closely associated with lactate steady state in the muscles during exercise.

Pavlat, Housh, Johnson, Schmidt, Eckerson (1993)

An examination of the electromyographic fatigue threshold

The purpose of this investigation was to compare times to exhaustion at various percentages of the EMG_{FT}. Eight male subjects participated in the study and each completed two initial EMG_{FT} tests. EMG_{FT} values were determined according to the method described by Matsumoto and colleagues (1991); briefly, this involves participants completing four fatiguing bouts of exercise and plotting the rate of increase in EMG amplitude across these bouts to estimate the power output that would not result in fatigue. Test-retest reliability showed no significant differences between initial EMG_{FT} values from the two tests. After this initial testing the subjects completed 5 time to exhaustion trials at 85, 100, 115, 130 and 145% of their individual EMG_{FT} values. Mean times to exhaustion at these percentages of EMG_{FT} were 495, 225, 135, 94, and 72 seconds, respectively. Additionally, the EMG_{FT} overestimated the power outputs that could be sustained for 30 and 60 minutes by 42% and 52%, respectively. These results are similar to the overestimation of non-fatiguing power output by Housh and colleagues (1991), who used the physical working capacity at neuromuscular fatigue threshold (PWC_{FT}) method, which is theoretically analogous to EMG_{FT}.

Housh, deVries, Johnson, Housh, Stout, Evetovich, Bradway (1995)

Electromyographic fatigue thresholds of the superficial muscles of the quadriceps femoris

The purpose of this study was to compare EMG_{FT} thresholds determined from each of the three superficial muscles of the quadriceps femoris (vastus lateralis: VL,

rectus femoris: RF, and vastus medialis: VM) using EMG data collected from all three muscles simultaneously. Because fiber type distribution varies from muscle to muscle, using EMG_{FT} from one muscle in a group (such as the quadriceps) may not accurately reflect what is occurring in terms of neuromuscular fatigue for the muscle group as a whole. Eight healthy, male and female college students volunteered to perform one EMG_{FT} test with electrodes placed on the VL, RF, and VM muscles. The results of this study indicated that there was a significant difference among mean EMG_{FT} values for VL and RF; further analysis showed that EMG_{FT} for RF was significantly lower than for VL. Therefore, EMG_{FT} determined from the VL may not accurately predict sustainable power output during cycle ergometry because the RF may fatigue at a lower power output.

Smith, Moon, Kendall, Graef, Lockwood, Walter, Beck, Cramer, Stout (2009)

The effects of β-alanine supplementation and high-intensity interval training on neuromuscular fatigue and muscle function.

The purpose of this study was to examine the effects of β-alanine supplementation and HIIT on neuromuscular fatigue, as measured using the EMG_{FT} method described by Matsumoto and colleagues (1991) and Moritani and colleagues (1993), and changes in the efficiency of electrical activity (EEA). This was the first study to use EMG_{FT} to report changes following training, although other studies have closely tied it to the aerobic-anaerobic transition phase of exercise and other anaerobic thresholds such as lactate and ventilatory thresholds. Forty-six men participated in the study and at PRE, MID, and POST testing they were asked to complete a $\dot{V}O_{2PEAK}$ test as well as four 2-minute work bouts on a cycle ergometer, during which EMG data was collected to

determine EMG_{FT} and EEA. The training consisted of two 3-week periods for a total of 6 weeks. Over the 6-week time period, the control group (CON, n = 10) was asked to continue their typical daily routines and the training groups completed three HIIT sessions per week, which ranged from 90-115% of each individual's peak power output during a $\dot{V}O_{2PEAK}$ test. The supplement group (BA n = 18) consumed 1.5 g of β-alanine four times per day for the first 3-week period followed by 1.5 g twice per day for the second 3-week period; meanwhile the placebo group (PL, n = 18) consumed an equivalent amount of dextrose. At MID, the training groups (BA and PL) improved both EMG_{FT} and EEA significantly, while CON did not change; similarly, at POST both training groups had improved EMG_{FT} and EEA relative to PRE. However, the authors reported no significant differences between BA and PL and posited that HIIT was the primary stimulus for improvements in measures of neuromuscular fatigue, rather than intramuscular carnosine content.

CHAPTER III: METHODS

Subjects

Forty recreationally active young men and women (men = 22, women = 18) were recruited to participate in the study. Three female participants were excluded due to issues with electromyography data collection, therefore data for 22 men and 15 women were included in the final analysis.

Following an explanation of the experimental protocol and all related benefits and risks, signed an informed consent. Health, activity levels, and prior supplementation of participants were assessed using a physical activity readiness questionnaire (PAR-Q) and a health & activity history questionnaire to determine participants' readiness for physical activity and whether any supplements they were already taking might interfere with the results of the study. All study protocols were approved by the New England Institutional Review Board. All participants signed an informed consent form prior to any data collection.

Experimental Design

A double blind, placebo controlled design, stratified for gender, was used to examine the effects of HIIT and HMB_{FA} on neuromuscular fatigue. Each participant was required to visit the Human Performance Laboratory at University of Central Florida twice for pre- and post- testing, with each testing session occurring on nonconsecutive days. The same testing protocols were repeated at the end of the four week training period. On the first testing day, anthropometric measures of participants were collected, including age (years, y), height (cm), and body mass (kg). Each participant then

performed a graded exercise test (GXT) to determine physical working capacity at neuromuscular fatigue threshold (PWC_{FT}). The peak wattage achieved during this test (P_{PEAK}) was used to establish individual training intensity. On the second day of testing, a fasted baseline blood draw was performed to measure serum HMB.

After baseline testing, the participants were randomly assigned to one of three groups: a control group (C), a HIIT with placebo group (P), and a HIIT with supplementation group (S). Of the 37 participants included in this study, 9 subjects were assigned to C and 14 to each of the training groups (P or S). In addition, participants were stratified for gender in each group.

High Intensity Interval Training (HIIT)

Participants assigned to P and S groups completed 12 HIIT sessions while participants assigned to C were asked to continue their normal activity patterns for the same duration before returning for post testing. HIIT sessions were scheduled three times per week on nonconsecutive days over a four week period and were performed on an electronically braked cycle ergometer (Corival, Lode; Groningen, Netherlands).

Participants began each session with a five minute warm-up cycling at a self-selected power output, followed by five two-minute bouts of work at a predetermined work load followed by one minute of rest. Training intensity varied by exercise session from submaximal to supramaximal following an undulating fractal pattern, ranging from 85-120% (Figure 1). Each individual participant's work load was calculated using his or her P_{PEAK} during the GXT at pretesting. If a participant was unable to complete an assigned work bout, the amount of time completed was recorded and the one minute rest

period began immediately. All training sessions took place under the supervision of a National Strength and Conditioning Association Certified Strength & Conditioning Specialist or an American College of Sports Medicine Health Fitness Specialist.

Supplementation

Each serving of the HMB_{FA} supplement consisted of 1 gram of β-hydroxy-β-methylbutyric free acid, reverse osmosis water, de-bittering agent, orange flavor, stevia extract, and potassium carbonate; each serving of placebo (PL) contained 1 gram of polydextrose that was equivalent to the β-hydroxy-β-methylbutyric free acid, citric acid, corn syrup, 10% stevia powder, de-bittering agent, and orange flavoring. The HMB_{FA} and PL were obtained from Metabolic Technologies Inc. (Ames, IA). Prior to the exercise session, subjects in S and P groups received 3 g per day of HMB_{FA} or PL (respectively) divided equally into three servings taken 30 minutes prior to exercise, again 1 hour later, and the final 1 g dose 3 hours post-exercise. To ensure compliance, investigators observed the subjects consume the supplement prior to and immediately after each exercise session. On the non-training days, subjects were instructed to consume one packet with three separate meals throughout the day. Empty packets were presented to the investigators upon returning to the laboratory following non-training days. In addition, blood plasma HMB concentrations were analyzed by gas chromatography-mass spectrometry which was performed by Metabolic Technologies Inc. in a blinded fashion using methods previously described by Nissen and colleagues (1990); the resulting HMB concentrations have previously been reported (Robinson IV et al., 2014).

Dietary Analysis

Prior to training, participants were asked to record as accurately as possible everything they consumed each day during three typical days (two weekdays and one weekend day). This diet was considered his or her standard diet and he or she was asked to replicate this style of diet throughout the study. These data were entered into a software program (Food Works 13, The Nutrition Company, Long Valley, NJ) which provided calculations of daily leucine intake (g) and total calories (kcal). The results of this analysis have previously been reported (Robinson IV et al., 2014).

Graded Exercise Test

Each participant performed an incremental test to volitional exhaustion on an electronically braked cycle ergometer (Excalibur Sport, Lode; Groningen, Netherlands) to determine the peak power output (P_{PEAK}) in watts (W), which was used to establish training load. The seat was adjusted so that the participant's legs were at near full extension during each pedal revolution and the participants were asked to maintain a pedal cadence of 70-75 rev·min^{-1} throughout the test. The participants began pedaling at 75 W and the power output was increased by 25 W every two minutes until volitional fatigue or until the participant was unable to maintain a pedal cadence of 70 rev·min^{-1} for ~10 seconds, despite strong verbal encouragement.

Electromyography (EMG) Measurements

A bipolar (4.6 cm center-to-center) surface electrode (Quinton Quick-Prep silver-silver chloride) arrangement was placed over the vastus lateralis muscle of the right thigh. Prior to electrode placement, the skin at each site was shaved and cleaned with alcohol.

The EMG electrodes were placed based on the recommendations from the SENIAM project for EMG electrode placement (Hermens et al., 1999). Inter-electrode impedance was kept below 5,000 ohms with abrasion of the skin beneath the electrodes. The raw EMG signal was sampled at 1 kHz, differentially amplified (EMG 100c, bandwidth = 10-500Hz, gain: x1000; MP150 Biopac Systems, Inc., Santa Barbara, CA), and digitally bandpass filtered (zero-phase shift fourth-order Butterworth) at 10-500 Hz. The EMG signals were expressed as root mean square (RMS) amplitude values (μVrms) and were recorded and stored on a personal computer (Dell Latitude E6530, Dell Inc., Round Rock, TX) for off-line analysis.

Determination of Physical Working Capacity at Neuromuscular Fatigue Threshold (PWC_{FT})

Physical working capacity at neuromuscular fatigue threshold (PWC_{FT}) values were determined using a D_{MAX} method similar to that which was presented by Bergstrom et al. (2011); test-retest reliability for this PWC_{FT} resulted in an intra-class correlation coefficient ($ICC_{3,1}$) of 0.95 (MD: 17 W; SEM: 6 W). In the present study, RMS values were calculated for each 10-second epoch during the GXT. Further, the 10-second RMS values were used to create moving averages for successive groups of $m = 3$ observations in an effort to reduce variability (Fox, 2000). The averaged RMS values were plotted against the midpoint of each 30-second window (Figure 1). The warmup values were excluded and the remaining data points were used to generate a third order polynomial regression representing the increase in amplitude versus time of the GXT (Figure 2).

The onset of fatigue (T_F) was defined as the x-value (time in seconds) of the point on the third order polynomial regression that measured the maximal perpendicular distance from the line between the first and last data points (Figure 1). T_F was used to estimate PWC_{FT} according to the following equation (Berthon & Fellmann, 2002; Zuniga et al., 2013):

$$PWC_{FT} = P_o + \alpha\left(\frac{n}{N}\right)$$

where P_O is the power output of the stage in which T_F occurred, α is the increment in power output between GXT stages (25 W), n is the difference in seconds between T_F and the beginning of the stage (s) during which T_F occurred, and N is the duration of a stage in seconds (120 s). Therefore, because the initial workload is 75 W, the equation can be rearranged to:

$$PWC_{FT} = 75 + 25 \times \left(\frac{T_F}{120}\right)$$

Statistical Analyses:

A two-way repeated measures analysis of variance [group (C, P, S) × time (PRE, POST)] was used to identify any group by time interaction for PWC_{FT} data. If a significant interaction occurred, the statistical model was decomposed by examining between groups with a one-way factorial ANOVA for each time point. Fisher's least significant difference (LSD) post hoc comparisons were performed between groups. If a significant main effect occurred, then dependent samples t-tests with Bonferroni corrections (p = [0.05/3] = 0.017) were performed across time for each group. For effect

size, partial eta squared statistic values were calculated, and according to Green and colleagues (2000), 0.01, 0.06, and 0.14 were interpreted as small, medium, and large effect sizes, respectively. An alpha of 0.05 was used to determine statistical significance. Data were analyzed via SPSS (Version 22.0, SPSS Inc., Chicago, IL).

CHAPTER IV: RESULTS

Figure 3 presents the mean + SD values for PWC_{FT} (W) at PRE and POST. The two-way ANOVA resulted in a significant interaction ($F = 6.69$, $p = 0.004$, $\eta 2 = 0.282$). Follow-up analysis with one-way ANOVA resulted in no difference among groups for pretest values ($F = 0.87$, $p = 0.43$), however a significant difference was found for posttest values ($F = 5.46$, $p = 0.009$). Post-hoc analysis among posttest values showed a significant difference between S vs P ($p = 0.034$) and C ($p = 0.003$). However, P was not significantly different from C ($p = 0.226$). In addition, the two-way ANOVA resulted in a significant main effect for time ($F = 14.095$, $p = 0.001$, $\eta 2 = 0.293$). Follow-up paired samples t-tests detected significant changes for S ($p < 0.001$) and P ($p = 0.016$), however, there was no change in C ($p = 0.473$).

CHAPTER V: DISCUSSION

To the best of our knowledge, this is the first study to examine the effects of the free acid form of β-hydroxy-β-methylbutyric free acid (HMB_{FA}) supplementation and cycle ergometer based high intensity interval training (HIIT) on physical working capacity at neuromuscular fatigue threshold (PWC_{FT}) in young men and women. The primary findings support the use of HIIT as a training method to increase PWC_{FT}. Furthermore, the results suggest that HMB_{FA} supplementation combined with four weeks of HIIT increases PWC_{FT} significantly more when compared with the placebo.

In general, the PWC_{FT} appears to be a valid and reliable technique for determining the onset of neuromuscular fatigue as characterized by an increase in EMG amplitude over time (deVries et al., 1987; deVries et al., 1989; Stout et al., 2007). Smith and colleagues (2009) were the first to examine the effects of HIIT on the onset of neuromuscular fatigue, using discontinuous, supramaximal workloads to determine EMG_{FT}. Although there is currently no research on the effects of HIIT on PWC_{FT}, it has been used to assess fitness (deVries et al., 1987), prescribe and evaluate the effectiveness of training programs to improve resistance to neuromuscular fatigue (deVries et al., 1989), and to evaluate the efficacy of supplements such as creatine, β-alanine, and arginine on delaying neuromuscular fatigue (Camic et al., 2010; Housh et al., 1991; Stout et al., 2006; Stout et al., 2007; Stout et al., 2000). In addition, the EMG_{FT} and PWC_{FT} reflect the same physiological limit on non-fatiguing work capacity, such that when power output exceeds these thresholds an increase in EMG amplitude over time is observed, signifying the onset of neuromuscular fatigue (deVries et al., 1982; deVries et

al., 1987). Therefore, it is relevant to discuss changes seen in EMG_{FT} when discussing changes in PWC_{FT}. In addition, several studies have reported significant relationships between EMG_{FT} or PWC_{FT} and other fatigue threshold measures such as ventilatory threshold (VT), respiratory compensation point (RCP), lactate threshold (LT), onset of blood lactate accumulation (OBLA), and critical power (CP) (Camic et al., 2014; deVries et al., 1982; Graef et al., 2008; Hug, Faucher, Kipson, & Jammes, 2003; Kendall et al., 2010; Lucia, Sanchez, Carvajal, & Chicharro, 1999; Maestu et al., 2006; Moritani et al., 1993; Nagata, Muro, Moritani, & Yoshida, 1981).

In the present study, the 4-week HIIT protocol resulted in a 10.4% and 15.8% increase in PWC_{FT} for placebo and HMB groups respectively, while a 2.9% decrease was observed in the control group. In support, Smith and colleagues (2009) reported a significant increase in EMG_{FT} (23.8%) after 3 weeks of training using a similar HIIT protocol while the control group demonstrated a non-significant 1.6% decrease in EMG_{FT}. Likewise, Laursen and colleagues (2002) reported 5% and 8% increases in VT and RCP, respectively, after only 2 weeks of HIIT in highly trained male cyclists. Moreover, Poole and colleagues (1985) reported 17% and 25% increases in LT and VT, respectively, after 4 weeks of HIIT in young sedentary males, while Helgerud and colleagues (2007) reported a 9-10% increase in LT after 8 weeks of HIIT in moderately trained males. Our results, along with previous studies, support the effectiveness of HIIT to elicit significant changes in several different fatigue threshold measures and suggest a common physiological adaptation to this method of exercise training.

The results of the current investigation suggest that HMB_{FA} supplementation in conjunction with HIIT results in a significantly greater increase in EMG_{FT} than training alone (Figure 3). These data are supported by Robinson and colleagues (2014) who reported a significantly greater increase in VT (14%) after 4 weeks of HIIT while supplementing with 3 grams per day of HMB_{FA}, compared to a 6% increase in the placebo group. Lamboley and colleagues (2007) also reported improvement in VT after 5 weeks of a running-based HIIT program with 3 grams per day of HMB_{Ca} supplementation or a placebo (11% and 9%, respectively), but the improvements were not significantly different among supplement and placebo groups. In contrast, Robinson and colleagues (2014) reported increases in RCP in both training groups with no significant difference between groups (HMB_{FA}: 7.6%; placebo: 9.9%), while Lamboley and colleagues (2007) reported significantly greater increases in RCP with HMB_{Ca} (13.4%) compared to placebo (8.3%).

HIIT has been shown to increase $\dot{V}O_{2PEAK}$, muscle buffering capacity, whole body fat oxidation, mitochondrial density, blood flow, and up-regulation of glycolytic enzymes (Burgomaster, Hughes, Heigenhauser, Bradwell, & Gibala, 2005; Edge et al., 2006; Henriksson, 1992; Krustrup, Söderlund, Mohr, & Bangsbo, 2004; Laursen et al., 2002; Little, Safdar, Wilkin, Tarnopolsky, & Gibala, 2010; Perry et al., 2008; Robinson IV et al., 2014; Weston, Zhou, Weatherby, & Robertson, 1997). Increases in oxidative capacity, fat oxidation, and mitochondrial density may be related to increases in Sirt1 and Sirt3, which are associated with increases in mitochondrial biogenesis (Verdin, Hirschey, Finley, & Haigis, 2010) via regulation of AMPK activation (Palacios et al., 2009) and the

action of AMPK on PGC-1α transcription (Cantó et al., 2009; Gerhart-Hines et al., 2007; Rodgers et al., 2005). In addition, Gibala and colleagues (2009) showed that brief, intense interval training activates AMPK, which may be the most important pathway for acute adaptations to exercise in skeletal muscle (Hardie & Sakamoto, 2006) and may be associated with increases in monocarboxylate transporter protein (MCT1 and MCT4) mRNA expression (Takimoto, Takeyama, & Hamada, 2013) and content (Perry et al., 2008) in skeletal muscle, possibly via expression of PGC-1α, which has a strong relationship with MCT1 (Thomas, Bishop, Lambert, Mercier, & Brooks, 2012). MCT1 and MCT4 are both membrane-bound sarcolemmal transport proteins involved in bidirectional lactate/H^+ cotransport, mediating most of the H^+ efflux during high intensity exercise; additionally MCT1 is found in skeletal muscle mitochondrial membranes and is important for shuttling lactate into the mitochondria for intracellular oxidation (Thomas et al., 2012). Increased expression of MCT proteins could contribute to the attenuation of intramuscular metabolic acidosis Recently, it has been suggested that HMB_{FA} may increase Sirt1, Sirt3, and AMPK activity in muscle cells (Bruckbauer et al., 2012), potentially increasing muscle oxidative capacity and consequently the power output at which the muscle begins relying on anaerobic metabolism,. HMB_{FA} may also enhance the effects of HIIT on PWC_{FT} by augmenting AMPK-modulated increases in MCT mRNA (Takimoto et al., 2013). In theory, PWC_{FT} represents the increased motor unit activation which leads to the accumulation of glycolytic metabolites such as lactate, hydrogen ions, inorganic phosphate, and ammonia (Moritani et al., 1993; Smith et al., 2007; Taylor, Bronks, & Bryant, 1997); therefore it follows that greater oxidative capacity and lactate/H^+ transport and lactate utilization may result in a delay of fatigue

and an increase in PWC_{FT}. In addition, HMB has been shown to decrease muscle protein degradation, increase muscle protein synthesis and muscle satellite cell activation, and may increase muscle regenerative capacity (Knitter et al., 2000; J. M. Wilson et al., 2013), but may be most effective when used following damage or when in a catabolic state (Nissen et al., 1996; G. J. Wilson et al., 2008). HMB_{FA} may act to enhance the effects of HIIT on PWC_{FT} by improving recovery between HIIT sessions, thereby allowing for greater improvements in $\dot{V}O_{2MAX}$ and lactate shuttling and utilization over time. Further research is needed to determine which, if any, of the proposed mechanisms mediate the interaction of HMB_{FA} with HIIT.

In summary, HIIT was effective in delaying the onset of neuromuscular fatigue as measured by PWC_{FT}, with HMB_{FA} enhancing this effect. While the mechanisms by which HMB_{FA} augmented the results of HIIT are unclear, they may include physiological adaptations such as improved recovery, increased mitochondrial biogenesis, and increased capacity to transport lactate and hydrogen ions out of the muscle or into mitochondria for oxidative metabolism.

APPENDIX A: TABLES

Table 1: Participant Descriptive Statistics

Variable	C (n = 9)	P (n = 14)	S (n = 14)
Age (y)	20.8 ± 1.9	23.5 ± 3.6	23.5 ± 3.6
Height (cm)	171.5 ± 9.1	171.7 ± 12.1	174.3 ± 8.6
Body Mass (kg)	76.4 ± 12.8	73.3 ± 17.0	74.6 ± 10.7

Data are presented as means ± SD. C = control; S = high intensity interval training with supplement; P = high intensity interval training with placebo. Supplement is 3 grams per day β-hydroxy-β-methylbutyric free acid (BetaTor™, Metabolic Technologies Inc, Ames, IA).

APPENDIX B: FIGURES

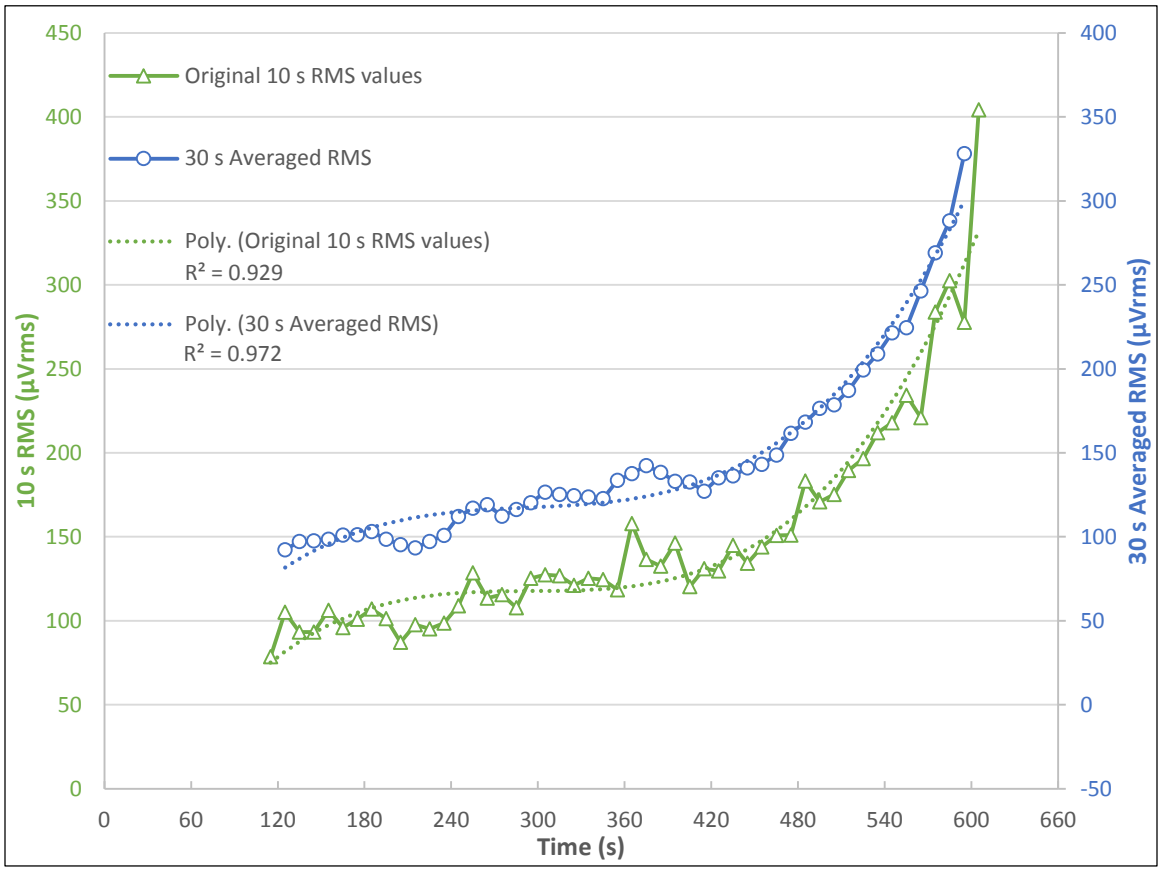

Figure 1: 30-second averaged RMS values.

The green triangles represent the original 10-second RMS values with the green dashed line representing the third-order polynomial regression of the original data ($R^2 = 0.929$). The blue circles represent averages of each possible set of three consecutive 10-second values with the dotted line representing the third-order polynomial regression of the smoothed data ($R^2 = 0.972$). The scales are offset to prevent overlap. RMS = root mean square (µVrms).

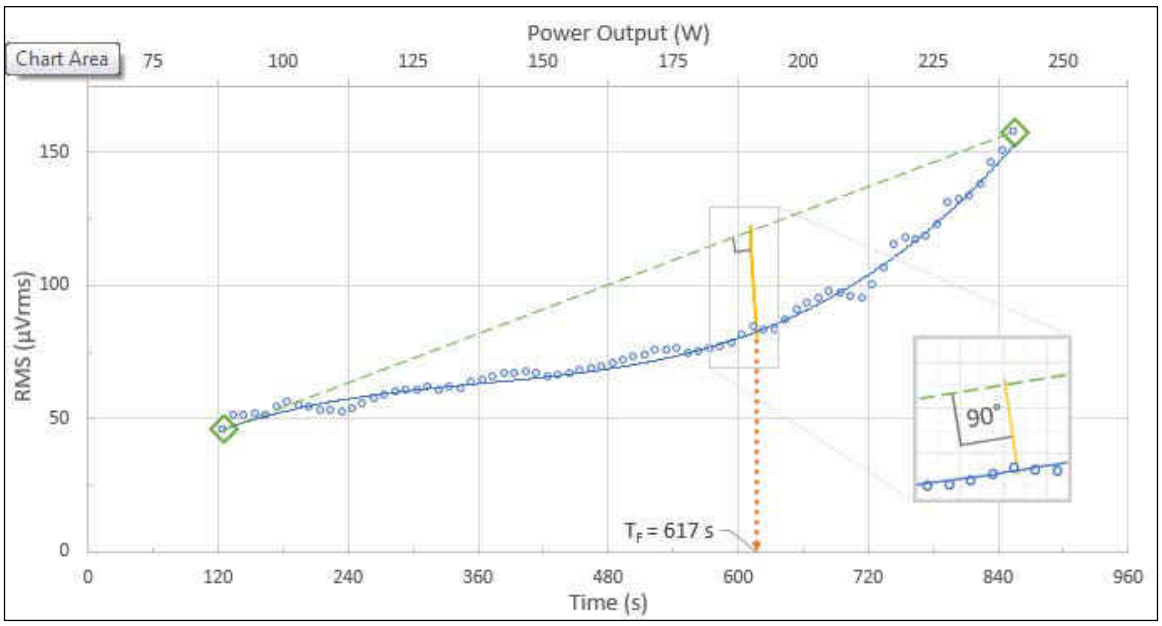

Figure 2: Example of determination of T_F using a D_{MAX} method.

The blue circles represent the smoothed data (using 30-second averages as demonstrated in Figure 1), and the solid blue curve represents the third-order polynomial regression of the smoothed data. The green diamonds mark the first and last data points included in the analysis and the dashed green line is a line between these two points. The yellow line represents the maximal perpendicular distance from the green line to the blue curve (D_{MAX}); the enlarged portion demonstrates that the green and yellow lines are indeed perpendicular. The orange dotted line represents determining T_F as the x-value (time in seconds) of the point on the blue curve at D_{MAX}. RMS = root mean square, (µVrms).

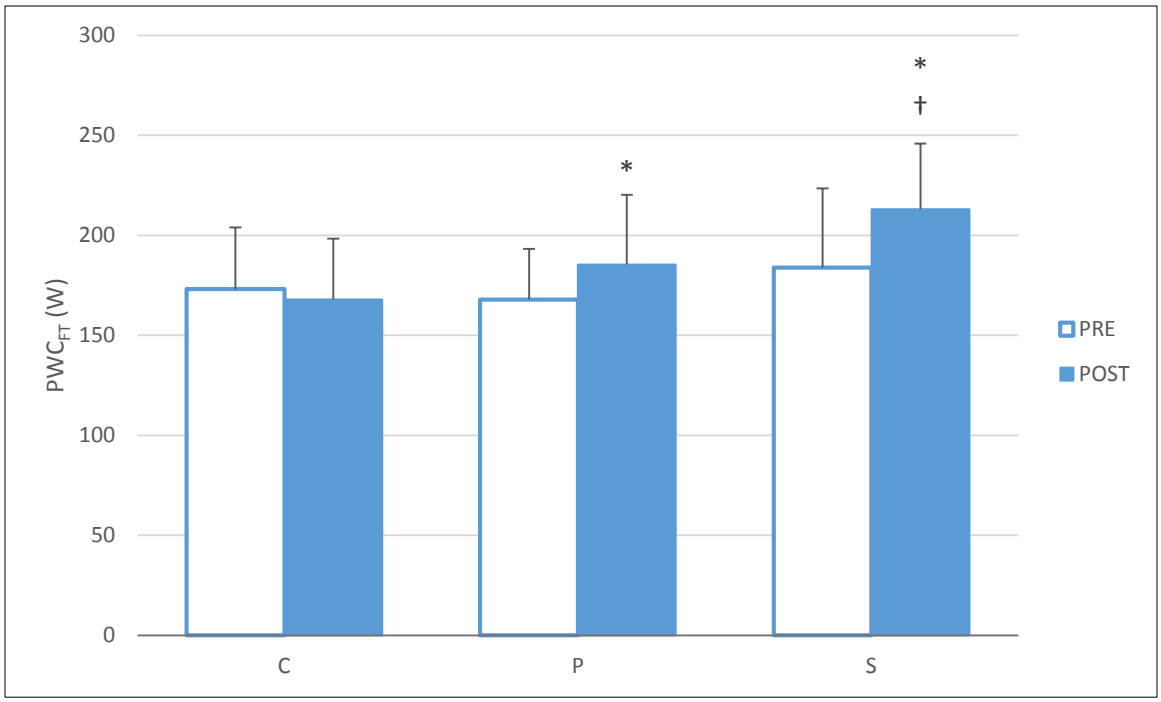

Figure 3: Physical working capacity at neuromuscular fatigue threshold (PWC_{FT}) by group (mean ± SD).

C = control. P = high intensity interval training with placebo. S = high intensity interval training with 3 grams per day of β-hydroxy-β-methylbutyric free acid. *Indicates significantly different from C ($p < 0.05$). †Indicates significantly different from P and C ($p < 0.05$).

APPENDIX C: UCF IRB LETTER

University of Central Florida

University of Central Florida Institutional Review Board
Office of Research & Commercialization
12201 Research Parkway, Suite 501
Orlando, Florida 32826-3246
Telephone: 407-823-2901, 407-882-2901 or 407-882-2276
www.research.ucf.edu/compliance/irb.html

Notice that UCF will Rely Upon Other IRB for Review and Approval

From : **UCF Institutional Review Board**
FWA00000351, IRB00001138

To : **Edward H. Robinson IV**

Date : **August 19, 2013**

IRB Number: **SBE-13-09475**

Study Title: **The effects of ß-Hydroxy-ß-methylbutyrate Free Acid Gel and High-Intensity Interval Training on Quadriceps Muscle Architecture and Quality, Neuromuscular Economy, and Metabolic Performance in Recreationally Trained Individuals**

Dear Researcher:

The research protocol noted above was reviewed by the University of Central Florida IRB Chair designated Reviewer on August 19, 2013. The UCF IRB accepts the New England Institutional Review Board's review and approval of this study for the protection of human subjects in research. **The expiration date will be the date assigned by the New England Institutional Review Board and the consent process will be the process approved by that IRB.**

This project may move forward as described in the protocol. It is understood that the New England IRB is the IRB of Record for this study, but local issues involving the UCF population should be brought to the attention of the UCF IRB as well for local oversight, if needed.

All data, including signed consent forms if applicable, must be retained in a locked file cabinet for a minimum of five years (six if HIPAA applies) past the completion of this research. Additional requirements may be imposed by your funding agency, your department, or other entities. Access to data is limited to authorized individuals listed as key study personnel.

Failure to provide a continuing review report for renewal of the study to the New England IRB could lead to study suspension, a loss of funding and/or publication possibilities, or a report of noncompliance to sponsors or funding agencies. If this study is funded by any branch of the Department of Health and Human Services (DHHS), an Office for Human Research Protections (OHRP) IRB Authorization form must be signed by the signatory officials of both institutions and a copy of the form must be kept on file at the IRB office of both institutions.

On behalf of Sophia Dziegielewski, Ph.D., L.C.S.W., UCF IRB Chair, this letter is signed by:

Signature applied by Patria Davis on 08/19/2013 10:41:19 AM EDT

IRB Coordinator

APPENDIX D: NEIRB APPROVAL

NEIRB
New England Institutional Review Board

August 13, 2013

Edward H. Robinson, IV
University of Central Florida
12494 University Boulevard
Orlando, FL 32816

Re: (IRB# 13-257): SBE-13-09475: "The Effects of β-Hydoxy- β-Methylbutyrate Free Acid Gel and High-Intensity Interval Training on Quadriceps Muscle Architecture and Quality, Neuromuscular Economy and Metabolic Performance in Recreationally Trained Individuals"

This is to inform you that New England Institutional Review Board (NEIRB), via expedited review (Thursday Board), has approved the above-referenced research protocol and the participation of the above-referenced investigative site in the research. The approval period is **8/13/2013 to 7/28/2014. Your study number is 13-257. Please be sure to reference either this number or the name of the principal investigator in any correspondence with NEIRB.**

Continued approval is conditional upon your compliance with the following requirements:

- A copy of the **Informed Consent Document**, NEIRB version 1.0, approved on 8/13/2013 is enclosed. Only NEIRB-approved informed consent documents should be used. It must be signed by each subject prior to initiation of any protocol procedures. In addition, each subject must be given a copy of the signed consent form.
- The following must be promptly reported to NEIRB: changes to the study site, and all unanticipated problems that may involve risks or affect the safety or welfare of subjects or others, or that may affect the integrity of the research.
- Approval is valid for enrollment of the number of subjects indicated on your submission form. If you anticipate enrolling more than this number of subjects, NEIRB approval must be obtained prior to exceeding the approved enrollment number.
- All protocol amendments and changes to approved research must be submitted to the IRB and not be implemented until approved by the IRB except where necessary to eliminate apparent immediate hazards to the study subjects.
- Compliance with all federal and state laws pertaining to this research, and with NEIRB's SOPs.
- The enclosed subject materials (*PAR-Q and You Questionnaire and QConfidential Medical and Acitvity History uestionnaire*) have been approved. The enclosed recruitment advertisement (*Print Ad*) has been conditionally approved. Please make the indicated revisions and re-submit it to NEIRB for final approval. Advertisements, letters, internet postings and any other media for subject recruitment must be submitted to NEIRB and approved prior to use. Please refer to *NEIRB Guidelines for Recruitment and Advertising*, available at www.neirb.com.
- All deaths, life-threatening problems or serious or unexpected adverse events, *whether related to the study article or not*, must be reported to the IRB. The Serious Adverse Event Form is available at www.neirb.com.
- Any and all necessary FDA approvals must be received prior to your initiation of the trial. If this study is being conducted under an IDE, a copy of the FDA IDE approval letter must be submitted to NEIRB.
- The study cannot continue after 7/28/2014 until re-approved by NEIRB. A Study Renewal Report must be completed and returned to NEIRB prior to the expiration of the approval period.

85 Wells Avenue · Suite 107 · Newton, MA 02459 Phone: 617-243-3924 · Fax: 617-969-1310 www.neirb.com

NEIRB
New England Institutional Review Board

- When the study is completed, terminated, or if it is not being renewed - complete and submit a Study Completion Report to NEIRB. The Study Completion Report can be accessed via the NEIRB website at www.neirb.com.

Shana R. Ross

Shana R. Ross, MCJ, CIM, CIP
Lead Administrator

Copy: NEIRB Chair
Enclosures

85 Wells Avenue · Suite 107 · Newton, MA 02459 Phone: 617-243-3924 · Fax: 617-969-1310 www.neirb.com

APPENDIX E: INFORMED CONSENT

The effects of β-Hydroxy-β-methylbutyrate Free Acid Gel and High-Intensity Interval Training on Quadriceps Muscle Architecture and Quality, Neuromuscular Economy, and Metabolic Performance in Recreationally Trained Individuals

Informed Consent

Principal Investigator(s):	Edward H. Robinson IV, M.A./M.S. Jeffrey R. Stout, Ph.D.
Sponsor:	Metabolic Technologies Inc.
Investigational Site(s):	University of Central Florida College of Education and Human Performance Sport and Exercise Science

Introduction: Researchers at the University of Central Florida (UCF) study many topics. To do this we need the help of people who agree to take part in a research study. You are being asked to take part in a research study that will include 40 men and women at UCF. You have been asked to take part in this research study because you are an active young adult who routinely participates in recreationally training. You must be between 18 and 35 years of age to be included in this research study.

The principle investigators conducting the research are Edward H. Robinson IV, and Dr. Jeffrey R. Stout. They will be supported by Dr. Jay R. Hoffman, Dr. Maren S. Fragala (Sport and Exercise Science in the College of Education), and Dr. Leonardo Oliveira (Sports Medicine Physician at UCF and medical monitor of the study).

What you should know about a research study:

- Someone will explain this research study to you.
- A research study is something you volunteer for.
- Whether or not you take part is up to you.
- You should take part in this study only because you want to.
- You can choose not to take part in the research study.
- You can agree to take part now and later change your mind.
- Whatever you decide it will not be held against you.
- Feel free to ask all the questions you want before you decide.

1. **Purpose of the research study:**
   We will examine two factors in this study:
   1) How exercise that involves repeated short-to-long bouts of high-intensity exercise interspersed with recovery periods, also know as, high intensity interval training (HIIT) effects cardiovascular and muscular adaptations to this form of endurance training.
   2) How supplementation with the free acid form β-Hydroxy-β-methylbutyrate (HMB-FA)—a chemical found naturally in the body and in some of the foods that we eat—effects the cardiovascular and muscular adaptations to this form of endurance training.

Testing location and time requirements:
All testing will be conducted in the Human Performance Lab (HPL) in the College of Education and Human Performance building at the University of Central Florida. All measures and tests are conducted for research purposes only. The results will not be used to diagnose any illness or disease, and will not provide any meaningful information to your physician.

Time requirements: We expect that you will be in this research study for approximately 6 weeks and will consist of 17 visits to the HPL. The first visit will last approximately an hour, the second and third visits about an hour and a half, and the training visits, 3 per week for 4 weeks, will last less than 30 minutes, and the final two testing visits will last approximately an hour and a half.

What you will be asked to do in the study:

Upon being admitted to the study you will be assigned a subject number. Each subject number will be associated with one of three groups: a control group (CTL), an HIIT only group (HIIT) or a group which will take the amino acid metabolite HMB and perform HIIT (HMB-HIIT). Determination of the group associated with each subject

Approved by NEIRB on 8/13/2013
NEIRB ICF version 1.0

number will occur by randomization (similar to flipping a coin). Of the 40 subjects that will be recruited for the study, 10 subjects will be assigned to CTL and 15 to each of the training groups. You will be unable to change your assigned study group to a different study group.

Individuals assigned to CTL will undergo testing on visits 2 and 3. They will then be asked to continue their normal exercise routine for 4 weeks and will undergo post-testing (visits 16 and 17) after this time period. Participants in the HIIT and HMB-HIIT groups will be asked on training days, to consume 1 gram HMB-FA or placebo 30 min prior to training, 1gram HMB-FA or placebo 1 hour post training, and 1gram HMB-FA or placebo 3 hours post training. On non-training days, individuals will consume HMB-FA or placebo 3 times per day (8am, 12pm and 4pm).

Preliminary Visits (3):

Visit 1: You will be asked to read and sign this consent form before any study-related procedures are performed. During this first visit, the following will be done:
Complete the Physical Activity Readiness Questionnaire (PAR-Q)
Complete the self-reported medical and activity history questionnaire
Your age, race and gender will be collected
Your body measurements (height, weight) will be measured
You will be given a 3-day food log to complete prior to visit 3. The dietary intake on this food log will be considered your pre-testing diet and you will be asked to maintain this style of diet during all experimental trials.

Visit 2: The second visit will take place at least 24 hours following visit 1. On this visit:
You will have an ultrasound performed on the quadriceps in your leg. For this, you will be asked to lie flat on your back on an examination table with your legs extended. A lubricated probe will be placed over your thigh to collect information about your muscle (cross-sectional area, fascicle length, echo intensity, muscle thickness). These images will provide the ability to rate the quality of your muscle and how the muscle quality may change after the training intervention.
You will be outfitted with surface electrodes over the vastus lateralis muscles in your quadriceps to measure electromyography (EMG). You will also be asked to perform a maximal leg extension to record a maximal EMG signal. The EMG signal will also be collected during the VO_2peak testing.
You will also be asked to perform a VO_2max test, which will include pedaling on the cycle ergometer at increasing resistance until you can no longer continue. Expired gases will be collected via a mask to determine oxygen uptake, respiratory quotient, energy expenditure and ventilatory threshold.

Visit 3: The third visit will take place no sooner than 48hrs following visit 2. On this visit:
You will have a blood sample taken. The total volume of blood that will be obtained during this study will be <25 ml. To put the total volume of blood being drawn in proper perspective, one pint (475 ml) of blood is typically drawn when donating blood. All blood draws will be conducted under sterile conditions. As an additional

Approved by NEIRB on 8/13/2013
NEIRB ICF version 1.0

safeguard in preventing contamination new disposable gloves will be used for all blood draws. The discomforts associated with the blood drawing procedures are minimal, but sometimes bruising and infection may occur, and your arm might become sore. This soreness usually resolves in a few days. If it persists, contact your doctor.

All blood samples collected will be frozen until analysis. However, blood samples obtained will only be used for this specific study and any leftover blood will be discarded following analysis.

You will have a dual energy x-ray absorptiometry (DEXA) scan performed to assess total and regional body composition. The DEXA machine consists of a padded table with a mechanical arm that uses low dose x-ray to measure muscle, adipose and bone mass. You will be asked to lie flat on your back, with your arms at your sides, legs extended and feet together. The mechanical arm of the DEXA will then pass slowly over your body, without contact. The full body scan will last about 15 minutes.
You will perform a 3-minute critical power test. After a self-selected warm-up, you will begin with 60 seconds of unloaded cycling at 90 rpm, followed by an all-out three-minute effort with resistance being set as a function of pedaling rate. The resistance will be adjusted during the all-out effort using the linear mode on the cycle ergometer that sets the power output at 50% of the difference between the ventilator threshold and peak power output assessed during the graded exercise test. EMG assessment will also be conducted during this test, electrode placement will be the same as previously described.

Training Visits (12):

If you are in the HIIT or HMB-HIIT group, you will complete 4 weeks of high intensity interval training (HIIT). The training will occur in the Human Performance Lab 3 times per week for 4 weeks with alternating training sessions of sub maximal and supramaximal workloads. Your training load will be determined as a percentage of the peak power output from the graded exercise test. Each training session with a 5-minute warm up at 50 W, followed by a protocol of 5 or 6 2-minute exercise bouts (total time 15-17 minutes) at a predetermined percentage of VO_2peak. There will be 1 minute of complete rest in between exercise bouts during which you will be asked about your perceived readiness to continue exercise.

Post-training Testing visits (2):
These visits will mirror visit 2 and visit 3.

Funding for this study: This research study is being funded by Metabolic Technologies Inc.

Risks:
The risks involved with this study are minimal, but may include musculoskeletal injuries occurring during the training protocol. These injuries include muscle strains and pulls. However, the interval training portion of the study is similar to a hard

Approved by NEIRB on 8/13/2013
NEIRB ICF version 1.0

training session that experienced recreationally trained individuals have previously performed during training. The risks associated with the blood draw include some momentary pain at the time of the draw, but other discomfort should be minimal. It is also possible for a bruise to develop at the site or for individuals to report dizziness and faint after the blood is drawn. It is also rare, but possible to develop minor infections and pain after the blood draw. To minimize the risks, the skin area at the site of the blood draw will be cleaned and prepared with a disinfectant wipe before the hypodermic is inserted. In addition, the blood draw will occur while the participant is lying supine. There are no risks or discomforts associated with any of the ultrasound measures. Procedures such as DEXA used during this research study involve X-rays. However, the cumulative radiation exposure from these tests is considered small and is not likely to adversely affect you. Additionally all testing and training will be overseen by individuals certified in CPR and AED. An AED is located in the building where testing and training will occur.

You should report any discomforts or injuries to one of the principle investigators Edward Robinson, 407-823-2367, ned.robinson@ucf.edu or Dr. Jeff Stout, 407-823-2367, jeffrey.stout@ucf.edu.

Benefits
There are no direct benefits to participants.

Compensation or payment:
Upon completion of the study, you will receive a $100 payment for participation. No compensation will be provided if you are unable to complete the study.

Confidentiality: The results of this study will be published as a group as part of a scientific publication. No individual results will be published or shared with any person or party. All information attained from the medical and activity questionnaire or performance tests will be held in strict confidence. Individual results will remain confidential and only be relayed to the subject upon request. All medical and activity questionnaires, as well as data collection sheets will be kept in a locked cabinet during and following the study. All information will be destroyed 5 years from the end of the study and not used for other research purposes. Participant folders and blood storage tubes will be marked with an I.D. number to protect against a breach of confidentiality and the ID number will be removed upon disposal. Participant names and I.D. numbers will be stored apart from the blood samples; the identifiers will be removed from the samples and destroyed when the samples are disposed.

Study contact for questions about the study or to report a problem: If you have questions, concerns, or complaints, or think the research has hurt you, please contact Ned Robinson or Dr. Jeff Stout, Human Performance Laboratory, Sport and Exercise Science (407) 823-2367 or by email at ned.robinson@ucf.edu or Jeffrey.stout@ucf.edu.

Approved by NEIRB on 8/13/2013
NEIRB ICF version 1.0

IRB contact about your rights in the study or to report a complaint: Research at the University of Central Florida involving human participants is carried out under the oversight of the New England Institutional Review Board (NEIRB). For information about the rights of people who take part in research, please contact: New England Institutional Review Board, at 1-800-232-9570. You may also talk to them for any of the following:

Your questions, concerns, or complaints are not being answered by the research team.
You cannot reach the research team.
You want to talk to someone besides the research team.
You want to get information or provide input about this research.

Withdrawing from the study:
You have the right to discontinue participation without penalty, regardless of the status of the study. Your participation in the study may also be terminated at any time by the researchers in charge of the project. This could be based upon your refusal to follow study instructions or follow the study protocol. Depending upon when you withdraw, you may be able to receive compensation for the time that you did participate. Please refer back to the "Compensation or Payment" section on the top of this page.

Approved by NEIRB on 8/13/2013
NEIRB ICF version 1.0

VOLUNTEER'S STATEMENT:

I have been given a chance to ask questions about this research study. These questions have been answered to my satisfaction. I may contact Edward Robinson if I have any more questions about taking part in this study. Edward Robinson or the company he/she is employed by is being paid by the sponsor for my participation in this study.

I understand that my participation in this research project is voluntary. I know that I may quit the study at any time without harming my future medical care or losing any benefits to which I might be entitled. I also understand that the investigator in charge of this study may decide at any time that I should no longer participate in this study.

If I have any questions about my rights as a research subject in this study I may contact:

New England Institutional Review Board
Telephone: 1-800-232-9570

By signing this form, I have not waived any of my legal rights.

I have read and understand the above information. I agree to participate in this study. I understand that I will be given a copy of this signed and dated form for my own records.

_____ _____
Study Participant (signature) Date

_____
Print Participant's Name

_____ _____
Person who explained this study (signature) Date

Approved by NEIRB on 8/13/2013
NEIRB ICF version 1.0

REFERENCES

Anthony, J. C., Anthony, T. G., Kimball, S. R., & Jefferson, L. S. (2001). Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine. *The Journal of Nutrition, 131*(3), 856S-860S. Retrieved from http://jn.nutrition.org/content/131/3/856S.full.pdf

Baptista, I. L., Leal, M. L., Artioli, G. G., Aoki, M. S., Fiamoncini, J., Turri, A. O., . . . Moriscot, A. S. (2010). Leucine attenuates skeletal muscle wasting via inhibition of ubiquitin ligases. *Muscle & Nerve, 41*(6), 800-808. doi:10.1002/mus.21578

Bergstrom, H. C., Zuniga, J. M., Housh, T. J., Camic, C. L., Traylor, D. A., Johnson, G. O., & Schmidt, R. J. (2011). Application of the dmax method to identify the gas exchange, ventilatory, and neuromuscular fatigue thresholds: 2158: Board #35, June 2, 2:00 PM-3:30 PM. *Medicine & Science in Sports & Exercise, 43*(5), 566. doi:10.1249/01.MSS.0000401558.43315.9a

Berthon, P., & Fellmann, N. (2002). General review of maximal aerobic velocity measurement at laboratory. Proposition of a new simplified protocol for maximal aerobic velocity assessment. *The Journal of Sports Medicine and Physical Fitness, 42*(3), 257-266. Retrieved from http://europepmc.org/abstract/med/12094113

Billat, L. V. (2001). Interval training for performance: A scientific and empirical practice. *Sports Medicine, 31*(1), 13-31. doi:00007256-200131020-00001

Bruckbauer, A., Zemel, M. B., Thorpe, T., Akula, M. R., Stuckey, A. C., Osborne, D., . . . Wall, J. S. (2012). Synergistic effects of leucine and resveratrol on insulin sensitivity and fat metabolism in adipocytes and mice. *Nutrition & Metabolism, 9*(1), 77-7075-9-77. doi:10.1186/1743-7075-9-77

Buchheit, M., & Laursen, P. B. (2013a). High-intensity interval training, solutions to the programming puzzle. Part I: Cardiopulmonary emphasis. *Sports Medicine, 43*, 313-338. doi:10.1007/s40279-013-0029-x

Buchheit, M., & Laursen, P. B. (2013b). High-intensity interval training, solutions to the programming puzzle. Part II: Anaerobic energy, neuromuscular load and practical applications. *Sports Medicine, 43*(10), 927-954. doi:10.1007/s40279-013-0066-5

Burgomaster, K. A., Howarth, K. R., Phillips, S. M., Rakobowchuk, M., MacDonald, M. J., McGee, S. L., & Gibala, M. J. (2008). Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *The Journal of Physiology, 586*(1), 151-160. doi:10.1113/jphysiol.2007.142109

Burgomaster, K. A., Hughes, S. C., Heigenhauser, G. J. F., Bradwell, S. N., & Gibala, M. J. (2005). Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *Journal of Applied Physiology (Bethesda, Md.: 1985), 98*(6), 1985-1990. doi:10.1152/japplphysiol.01095.2004

Camic, C. L., Housh, T. J., Zuniga, J. M., Hendrix, C. R., Mielke, M., Johnson, G. O., & Schmidt, R. J. (2010). Effects of arginine-based supplements on the physical working capacity at the fatigue threshold. *Journal of Strength and Conditioning Research, 24*(5), 1306-1312. doi:10.1519/JSC.0b013e3181d68816

Camic, C. L., Kovacs, A. J., Enquist, E. A., VanDusseldorp, T. A., Hill, E. C., Calantoni, A. M., & Yemm, A. J. (2014). An electromyographic-based test for estimating neuromuscular fatigue during incremental treadmill running. *Physiological Measurement, 35*(12), 2401. doi:10.1088/0967-3334/35/12/2401

Cantó, C., Gerhart-Hines, Z., Feige, J. N., Lagouge, M., Noriega, L., Milne, J. C., . . . Auwerx, J. (2009). AMPK regulates energy expenditure by modulating NAD metabolism and SIRT1 activity. *Nature, 458*(7241), 1056-1060. doi:10.1038/nature07813

Clark, I. E., West, B. M., Reynolds, S. K., Murray, S. R., & Pettitt, R. W. (2013). Applying the critical velocity model for an off-season interval training program. *Journal of Strength and Conditioning Research, 27*(12), 3335-3341. doi:10.1519/JSC.0b013e31828f9d87

Daussin, F. N., Zoll, J., Dufour, S. P., Ponsot, E., Lonsdorfer-Wolf, E., Doutreleau, S., . . . Richard, R. (2008). Effect of interval versus continuous training on cardiorespiratory and mitochondrial functions: Relationship to aerobic performance improvements in sedentary subjects. *American Journal of Physiology - Regulatory,*

Integrative and Comparative Physiology, 295(1), R264-72. doi:10.1152/ajpregu.00875.2007

deVries, H. A., Brodowicz, G. R., Robertson, L. D., Svoboda, M. D., Schendel, J. S., Tichy, A. M., & Tichy, M. W. (1989). Estimating physical working capacity and training changes in the elderly at the fatigue threshold (PWC_{FT}). *Ergonomics, 32*(8), 967-977. doi:10.1080/00140138908966858

deVries, H. A., Housh, T. J., Johnson, G. O., Evans, S. A., Tharp, G. D., Housh, D. J., & Hughes, R., A. (1990). Factors affecting the estimation of physical working capacity at the fatigue threshold. *Ergonomics, 33*(1), 25-33. doi:10.1080/00140139008927091

deVries, H. A., Moritani, T., Nagata, A., & Magnussen, K. (1982). The relation between critical power and neuromuscular fatigue as estimated from electromyographic data. *Ergonomics, 25*(9), 783-791. doi:10.1080/00140138208925034

deVries, H. A., Tichy, M. W., Housh, T. J., Smyth, K. D., Tichy, A. M., & Housh, D. J. (1987). A method for estimating physical working capacity at the fatigue threshold (PWC_{FT}). *Ergonomics, 30*(8), 1195-1204. doi:10.1080/00140138708966008

Edge, J., Bishop, D., & Goodman, C. (2006). The effects of training intensity on muscle buffer capacity in females. *European Journal of Applied Physiology, 96*(1), 97-105. doi:10.1007/s00421-005-0068-6

Emerson, N. S., Fukuda, D. H., Stout, J. R., Robinson, E. H., McCormack, W. P., Scanlon, T. C., . . . Mangine, G. T. (2014). Physical working capacity at fatigue

threshold (PWC_{FT}) is associated with sarcopenia-related body composition and measures of functionality in older adults. Archives of Gerontology and Geriatrics, 59(2), 300-304. doi:10.1016/j.archger.2014.04.012

Fox, J. (2000). *Nonparametric Simple Regression: Smoothing Scatterplots.* Sage University Papers Series on Quantitative Applications in the Social Sciences, 07-130. Thousand Oaks, CA: Sage.

Fuller, J. C., Sharp, R. L., Angus, H. F., Baier, S. M., & Rathmacher, J. A. (2011). Free acid gel form of β-hydroxy-β-methylbutyrate (HMB) improves HMB clearance from plasma in human subjects compared with the calcium HMB salt. *British Journal of Nutrition, 105*(03), 367-372. doi:10.1017/S0007114510003582

Gerhart-Hines, Z., Rodgers, J. T., Bare, O., Lerin, C., Kim, S., Mostoslavsky, R., . . . Puigserver, P. (2007). Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1α. *The EMBO Journal, 26*(7), 1913-1923. doi:10.1038/sj.emboj.7601633

Gibala, M. J., Little, J. P., Van Essen, M., Wilkin, G. P., Burgomaster, K. A., Safdar, A., . . . Tarnopolsky, M. A. (2006). Short-term sprint interval versus traditional endurance training: Similar initial adaptations in human skeletal muscle and exercise performance. *The Journal of Physiology, 575*(3), 901-911. doi:10.1113/jphysiol.2006.112094

Gibala, M. J., McGee, S. L., Garnham, A. P., Howlett, K. F., Snow, R. J., & Hargreaves, M. (2009). Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1α in human skeletal muscle. *Journal of Applied Physiology (Bethesda, Md.: 1985), 106*(3), 929-934. doi:10.1152/japplphysiol.90880.2008

Gonzalez, A. M., Stout, J. R., Jajtner, A. R., Townsend, J. R., Wells, A. J., Beyer, K. S., . . . Scanlon, T. M. (2014). Effects of β-hydroxy-β-methylbutyrate free acid and cold water immersion on post-exercise markers of muscle damage. *Amino Acids, 46*(6), 1501-1511. doi:10.1007/s00726-014-1722-2

Graef, J. L., Smith, A. E., Kendall, K. L., Walter, A. A., Moon, J. R., Lockwood, C. M., . . . Stout, J. R. (2008). The relationships among endurance performance measures as estimated from $\dot{V}O_{2PEAK}$, ventilatory threshold, and electromyographic fatigue threshold: A relationship design. *Dynamic Medicine, 7*, 15-5918-7-15. doi:10.1186/1476-5918-7-15

Green, S., Salkind, N., & Akey, T. (2000). Methods for controlling type I error across multiple hypothesis tests. *Using SPSS for windows: Analyzing and understanding data* (2nd ed., pp. 395-396). New Jersey: Prentice-Hall.

Hardie, D. G., & Sakamoto, K. (2006). AMPK: A key sensor of fuel and energy status in skeletal muscle. *Physiology (Bethesda, Md.), 21*, 48-60. doi:10.1152/physiol.00044.2005

Helgerud, J., Hoydal, K., Wang, E., Karlsen, T., Berg, P., Bjerkaas, M., . . . Bach, R. (2007). Aerobic high-intensity intervals improve $\dot{V}O_{2MAX}$ more than moderate training. *Medicine & Science in Sports & Exercise, 39*(4), 665-671. doi:10.1249/mss.0b013e3180304570

Henriksson, J. (1992). Effects of physical training on the metabolism of skeletal muscle. *Diabetes Care, 15*(11), 1701-1711. doi:10.2337/diacare.15.11.1701

Hermens, H. J., Freriks, B., Merletti, R., Stegeman, D., Blok, J., Rau, G., . . . Hägg, G. (1999). *European recommendations for surface electromyography* Roessingh Research and Development. The Netherlands.

Hoffman, J. R., Ratamess, N. A., Tranchina, C. P., Rashti, S. L., Kang, J., & Faigenbaum, A. D. (2010). Effect of a proprietary protein supplement on recovery indices following resistance exercise in strength/power athletes. *Amino Acids, 38*(3), 771-778. doi:10.1007/s00726-009-0283-2

Holecek, M., Muthny, T., Kovarik, M., & Sispera, L. (2009). Effect of β-hydroxy-β-methylbutyrate (HMB) on protein metabolism in whole body and in selected tissues. *Food and Chemical Toxicology, 47*(1), 255-259. doi:10.1016/j.fct.2008.11.021

Housh, T. J., deVries, H. A., Johnson, G. O., & Evans, S. A. (1991). An evaluation of the physical working capacity at the fatigue threshold test. *Medicine & Science in Sports & Exercise, 23*, S94.

Housh, T. J., Johnson, G. O., Evans, S. A., & McDowell, S. (1991). The effect of ammonium chloride and sodium bicarbonate ingestion on the physical working capacity at the fatigue threshold. *European Journal of Applied Physiology and Occupational Physiology, 62*(3), 189-192. doi:10.1007/BF00643740

Housh, T. J., Johnson, G. O., Housh, D. J., Evans, S. A., Stout, J. R., Evetovich, T. K., & Bradway, R. M. (1995). Electromyographic fatigue thresholds of the superficial muscles of the quadriceps femoris. *European Journal of Applied Physiology and Occupational Physiology, 71*(2-3), 131-136. doi:10.1007/BF00854969

Hug, F., Faucher, M., Kipson, N., & Jammes, Y. (2003). EMG signs of neuromuscular fatigue related to the ventilatory threshold during cycling exercise. *Clinical Physiology and Functional Imaging, 23*(4), 208-214. doi:10.1046/j.1475-097X.2003.00497.x

Jenkins, D. G., & Quigley, B. M. (1993). The influence of high-intensity exercise training on the wlim-tlim relationship. *Medicine & Science in Sports & Exercise, 25*(2), 275-282. doi:10.1249/00005768-199302000-00019

Jourkesh, M., Ahmaidi, S., Keikha, B. M., Sadri, I., & Ojagi, A. (2011). Effects of six weeks sodium bicarbonate supplementation and high-intensity interval training on endurance performance and body composition. *Annals of Biological Research, 2*(2), 403-413. Retrieved from http://scholarsresearchlibrary.com/ABR-vol2-iss2/ABR-2011-2-2-403-413.pdf

Kendall, K. L., Smith, A. E., Graef, J. L., Walter, A. A., Moon, J. R., Lockwood, C. M., . . . Stout, J. R. (2010). Validity of electromyographic fatigue threshold as a noninvasive method for tracking changes in ventilatory threshold in college-aged men. *Journal of Strength and Conditioning Research, 24*(1), 109-113. doi:10.1519/JSC.0b013e31819b79bc

Knitter, A. E., Panton, L., Rathmacher, J. A., Petersen, A., & Sharp, R. (2000). Effects of β-hydroxy-β-methylbutyrate on muscle damage after a prolonged run. *Journal of Applied Physiology (Bethesda, Md.: 1985), 89*(4), 1340-1344. Retrieved from http://jap.physiology.org/content/jap/89/4/1340.full.pdf

Koopman, R., Wagenmakers, A. J. M., Manders, R. J. F., Zorenc, A. H. G., Senden, J. M. G., Gorselink, M., . . . van Loon, L. J. C. (2005). Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. *American Journal of Physiology - Endocrinology and Metabolism, 288*(4), E645-53. doi:10.1152/ajpendo.00413.2004

Krustrup, P., Söderlund, K., Mohr, M., & Bangsbo, J. (2004). The slow component of oxygen uptake during intense, sub-maximal exercise in man is associated with additional fibre recruitment. *Pflügers Archiv European Journal of Physiology, 447*(6), 855-866. doi:10.1007/s00424-003-1203-z

Lamboley, C. R., Royer, D., & Dionne, I. J. (2007). Effects of β-hydroxy-β-methylbutyrate on aerobic-performance components and body composition in college students. *International Journal of Sport Nutrition & Exercise Metabolism,*

17(1), 56-69. Retrieved from http://journals.humankinetics.com/AcuCustom/Sitename/Documents/DocumentItem/6695.pdf

Laursen, P. B., Blanchard, M. A., & Jenkins, D. G. (2002). Acute high-intensity interval training improves tvent and peak power output in highly trained males. *Canadian Journal of Applied Physiology, 27*(4), 336-348. doi:10.1139/h02-019

Laursen, P. B., & Jenkins, D. G. (2002). The scientific basis for high-intensity interval training. *Sports Medicine, 32*(1), 53-73. doi:10.2165/00007256-200232010-00003

Laursen, P. B., Shing, C. M., Peake, J. M., Coombes, J. S., & Jenkins, D. G. (2005). Influence of high-intensity interval training on adaptations in well-trained cyclists. *Journal of Strength and Conditioning Research, 19*(3), 527-533. Retrieved from http://pdfs.journals.lww.com/nsca-jscr/2005/08000/INFLUENCE_OF_HIGH_INTENSITY_INTERVAL_TRAINING_ON.8.pdf

Little, J. P., Safdar, A., Wilkin, G. P., Tarnopolsky, M. A., & Gibala, M. J. (2010). A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: Potential mechanisms. *The Journal of Physiology, 588*(6), 1011-1022. doi:10.1113/jphysiol.2009.181743

Lucia, A., Sanchez, O., Carvajal, A., & Chicharro, J. L. (1999). Analysis of the aerobic-anaerobic transition in elite cyclists during incremental exercise with the use of

electromyography. *British Journal of Sports Medicine, 33*(3), 178-185. doi:10.1136/bjsm.33.3.178

Mäestu, J., Cicchella, A., Purge, P., Ruosi, S., Jurimae, J., & Jurimae, T. (2006). Electromyographic and neuromuscular fatigue thresholds as concepts of fatigue. *Journal of Strength and Conditioning Research, 20*(4), 824-828. doi:10.1519/R-18275.1

Matsumoto, T., Ito, K., & Moritani, T. (1991). The relationship between anaerobic threshold and electromyographic fatigue threshold in college women. *European Journal of Applied Physiology and Occupational Physiology, 63*(1), 1-5. doi:10.1007/BF00760792

McCormack, W. P., Stout, J. R., Emerson, N. S., Scanlon, T. C., Warren, A. M., Wells, A. J., . . . Fragala, M. S. (2013). Oral nutritional supplement fortified with β-alanine improves physical working capacity in older adults: A randomized, placebo-controlled study. *Experimental Gerontology, 48*(9), 933-939. doi:10.1016/j.exger.2013.06.003

Mohr, M., Krustrup, P., Nielsen, J. J., Nybo, L., Rasmussen, M. K., Juel, C., & Bangsbo, J. (2007). Effect of two different intense training regimens on skeletal muscle ion transport proteins and fatigue development. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 292*(4), R1594-602. doi:10.1152/ajpregu.00251.2006

Moritani, T., Takaishi, T., & Matsumoto, T. (1993). Determination of maximal power output at neuromuscular fatigue threshold. *Journal of Applied Physiology (Bethesda, Md.: 1985), 74*(4), 1729-1734. Retrieved from http://jap.physiology.org/content/74/4/1729.full.pdf

Moritani, T., & Yoshitake, Y. (1998). 1998 ISEK Congress Keynote Lecture: The use of electromyography in applied physiology. International Society of Electrophysiology and Kinesiology. *Journal of Electromyography and Kinesiology, 8*(6), 363-381. doi:10.1016/S1050-6411(98)00018-2

Nagata, A., Muro, M., Moritani, T., & Yoshida, T. (1981). Anaerobic threshold determination by blood lactate and myoelectric signals. *The Japanese Journal of Physiology, 31*(4), 585-597. doi:10.2170/jjphysiol.31.585

Nissen, S. L., & Abumrad, N. N. (1997). Nutritional role of the leucine metabolite β-hydroxy β-methylbutyrate (HMB). *The Journal of Nutritional Biochemistry, 8*(6), 300-311. doi:10.1016/S0955-2863(97)00048-X

Nissen, S. L., Ray, M., Rathmacher, J. A., Rice, D., Fuller, J. C., Jr., Connelly, A. S., & Abumrad, N. N. (1996). Effect of leucine metabolite β-hydroxy-β-methylbutyrate on muscle metabolism during resistance-exercise training. *Journal of Applied Physiology (Bethesda, Md.: 1985), 81*(5), 2095-2104. Retrieved from http://jap.physiology.org/content/jap/81/5/2095.full.pdf

Nissen, S. L., Sharp, R. L., Panton, L. B., Vukovich, M. D., Trappe, S. W., & Fuller, J. C., Jr. (2000). B-hydroxy-β-methylbutyrate (HMB) supplementation in humans is safe and may decrease cardiovascular risk factors. *The Journal of Nutrition, 130*(8), 1937-1945. Retrieved from http://jn.nutrition.org/content/130/8/1937.full.pdf

Nissen, S. L., Van Koevering, M., & Webb, D. (1990). Analysis of β-hydroxy-β-methylbutyrate in plasma by gas chromatography and mass spectrometry. *Analytical Biochemistry, 188*(1), 17-19. Retrieved from http://www.sciencedirect.com/science/article/pii/000326979090522B

O'Connor, D. M., & Crowe, M. J. (2003). Effects of β-hydroxy-β-methylbutyrate and creatine monohydrate supplementation on the aerobic and anaerobic capacity of highly trained athletes. *The Journal of Sports Medicine and Physical Fitness, 43*(1), 64-68. Retrieved from http://europepmc.org/abstract/med/12629464

Palacios, O. M., Carmona, J. J., Michan, S., Chen, K. Y., Manabe, Y., Ward, J. L.,3rd, . . . Tong, Q. (2009). Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1α in skeletal muscle. *Aging, 1*(9), 771-783. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2815736/pdf/aging-01-771.pdf

Panton, L. B., Rathmacher, J. A., Baier, S. M., & Nissen, S. L. (2000). Nutritional supplementation of the leucine metabolite β-hydroxy-β-methylbutyrate (HMB) during resistance training. *Nutrition, 16*(9), 734-739. doi:10.1016/S0899-9007(00)00376-2

Park, B., Henning, P. C., Grant, S. C., Lee, W. J., Lee, S., Arjmandi, B. H., & Kim, J. (2013). HMB attenuates muscle loss during sustained energy deficit induced by calorie restriction and endurance exercise. *Metabolism, 62*(12), 1718-1729. doi:10.1016/j.metabol.2013.06.005

Pavlat, D. J., Housh, T. J., Johnson, G. O., Schmidt, R. J., & Eckerson, J. M. (1993). An examination of the electromyographic fatigue threshold test. *European Journal of Applied Physiology and Occupational Physiology, 67*(4), 305-308. doi:10.1007/BF00357627

Perry, C. G., Heigenhauser, G. J., Bonen, A., & Spriet, L. L. (2008). High-intensity aerobic interval training increases fat and carbohydrate metabolic capacities in human skeletal muscle. *Applied Physiology, Nutrition, and Metabolism, 33*(6), 1112-1123. doi:10.1139/H08-097

Pinheiro, Carlos Hermano da Justa, Gerlinger-Romero, F., Guimaraes-Ferreira, L., de Souza-Jr, A. L., Vitzel, K. F., Nachbar, R. T., . . . Curi, R. (2012). Metabolic and functional effects of β-hydroxy-β-methylbutyrate (HMB) supplementation in skeletal muscle. *European Journal of Applied Physiology, 112*(7), 2531-2537. doi:10.1007/s00421-011-2224-5

Poole, D. C., & Gaesser, G. A. (1985). Response of ventilatory and lactate thresholds to continuous and interval training. *Journal of Applied Physiology, 58*(4), 1115-1115-1121. Retrieved from http://jap.physiology.org/content/58/4/1115.full.pdf

Robinson IV, E. H., Stout, J. R., Miramonti, A. A., Fukuda, D. H., Wang, R., Townsend, J. R., . . . Hoffman, J. R. (2014). High-intensity interval training and β-hydroxy-β-methylbutyric free acid improves aerobic power and metabolic thresholds. *Journal of the International Society of Sports Nutrition, 11*(1), 16. doi:10.1186/1550-2783-11-16

Rodgers, J. T., Lerin, C., Haas, W., Gygi, S. P., Spiegelman, B. M., & Puigserver, P. (2005). Nutrient control of glucose homeostasis through a complex of PGC-1α and SIRT1. *Nature, 434*(7029), 113-118. doi:10.1038/nature03354

Slater, G. J., & Jenkins, D. (2000). B-hydroxy-β-methylbutyrate (HMB) supplementation and the promotion of muscle growth and strength. *Sports Medicine, 30*(2), 105-116. doi:10.2165/00007256-200030020-00004

Slater, G., Jenkins, D., Logan, P., Lee, H., Vukovich, M., Rathmacher, J. A., & Hahn, A. G. (2001). B-hydroxy-β-methylbutyrate (HMB) supplementation does not affect changes in strength or body composition during resistance training in trained men. *International Journal of Sport Nutrition and Exercise Metabolism, 11*(3), 384-396. Retrieved from http://europepmc.org/abstract/med/11599506

Smith, A. E., Moon, J. R., Kendall, K. L., Graef, J. L., Lockwood, C. M., Walter, A. A., . . . Stout, J. R. (2009). The effects of β-alanine supplementation and high-intensity interval training on neuromuscular fatigue and muscle function. *European Journal of Applied Physiology, 105*(3), 357-363. doi:10.1007/s00421-008-0911-7

Smith, A. E., Walter, A. A., Graef, J. L., Kendall, K. L., Moon, J. R., Lockwood, C. M., . . . Stout, J. R. (2009). Effects of β-alanine supplementation and high-intensity interval training on endurance performance and body composition in men; a double-blind trial. *Journal of the International Society of Sports Nutrition, 6*(1), 1-9. doi:10.1186/1550-2783-6-5

Smith, A. E., Walter, A. A., Herda, T. J., Ryan, E. D., Moon, J. R., Cramer, J. T., & Stout, J. R. (2007). Effects of creatine loading on electromyographic fatigue threshold during cycle ergometry in college-aged women. *Journal of the International Society of Sports Nutrition, 4*(1), 1-6. doi:10.1186/1550-2783-4-20

Stout, J. R., Cramer, J. T., Mielke, M., O'Kroy, J., Torok, D., & Zoeller, R. F. (2006). Effects of twenty-eight days of β-alanine and creatine monohydrate supplementation on the physical working capacity at neuromuscular fatigue threshold. *Journal of Strength and Conditioning Research, 20*(4), 928-931. doi:10.1519/R-19655.1

Stout, J. R., Cramer, J. T., Zoeller, R. F., Torok, D., Costa, P., Hoffman, J. R., . . . O'Kroy, J. (2007). Effects of β-alanine supplementation on the onset of neuromuscular fatigue and ventilatory threshold in women. *Amino Acids, 32*(3), 381-386. doi:10.1007/s00726-006-0474-z

Stout, J., Eckerson, J., Ebersole, K., Moore, G., Perry, S., Housh, T., . . . Batheja, A. (2000). Effect of creatine loading on neuromuscular fatigue threshold. *Journal of Applied Physiology (Bethesda, Md.: 1985), 88*(1), 109-112. Retrieved from http://jap.physiology.org/content/88/1/109.full.pdf

Sun, X., & Zemel, M. B. (2009). Leucine modulation of mitochondrial mass and oxygen consumption in skeletal muscle cells and adipocytes. *Nutrition & Metabolism (London), 6*(1), 26. doi:10.1186/1743-7075-6-26

Takimoto, M., Takeyama, M., & Hamada, T. (2013). Possible involvement of AMPK in acute exercise-induced expression of monocarboxylate transporters MCT1 and MCT4 mRNA in fast-twitch skeletal muscle. *Metabolism, 62*(11), 1633-1640. doi:10.1016/j.metabol.2013.06.010

Talanian, J. L., Galloway, S. D., Heigenhauser, G. J., Bonen, A., & Spriet, L. L. (2007). Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women. *Journal of Applied Physiology (Bethesda, Md.: 1985), 102*(4), 1439-1447. doi:10.1152/japplphysiol.01098.2006

Tang, J. E., Moore, D. R., Kujbida, G. W., Tarnopolsky, M. A., & Phillips, S. M. (2009). Ingestion of whey hydrolysate, casein, or soy protein isolate: Effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *Journal of Applied Physiology (Bethesda, Md.: 1985), 107*(3), 987-992. doi:10.1152/japplphysiol.00076.2009

Taylor, A. D., Bronks, R., & Bryant, A. L. (1997). The relationship between electromyography and work intensity revisited: A brief review with references to lacticacidosis and hyperammonia. *Electromyography and Clinical Neurophysiology, 37*(7), 387-398. Retrieved from http://europepmc.org/abstract/med/9402427

Thomas, C., Bishop, D. J., Lambert, K., Mercier, J., & Brooks, G. A. (2012). Effects of acute and chronic exercise on sarcolemmal MCT1 and MCT4 contents in human skeletal muscles: Current status. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 302*(1), R1-14. doi:10.1152/ajpregu.00250.2011

Townsend, J. R., Fragala, M. S., Jajtner, A. R., Gonzalez, A. M., Wells, A. J., Mangine, G. T., . . . Hoffman, J. R. (2013). B-hydroxy-β-methylbutyrate (HMB)-free acid attenuates circulating TNF-α and TNFR1 expression postresistance exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985), 115*(8), 1173-1182. doi:10.1152/japplphysiol.00738.2013

Van Koevering, M., & Nissen, S. L. (1992). Oxidation of leucine and α-ketoisocaproate to β-hydroxy-β-methylbutyrate in vivo. *The American Journal of Physiology, 262*(1 Pt 1), E27-31. Retrieved from http://ajpendo.physiology.org/content/262/1/E27.full.pdf

Verdin, E., Hirschey, M. D., Finley, L. W., & Haigis, M. C. (2010). Sirtuin regulation of mitochondria: Energy production, apoptosis, and signaling. *Trends in Biochemical Sciences, 35*(12), 669-675. doi:10.1016/j.tibs.2010.07.003

Vukovich, M. D., & Dreifort, G. D. (2001). Effect of β-hydroxy-β-methylbutyrate on the onset of blood lactate accumulation and $\dot{V}O_{2PEAK}$ in endurance-trained cyclists. *The Journal of Strength & Conditioning Research, 15*(4), 491-497. doi:10.1519/00124278-200111000-00015

Weston, S. B., Zhou, S., Weatherby, R. P., & Robertson, S. J. (1997). Does exogenous coenzyme Q10 affect aerobic capacity in endurance athletes? *International Journal of Sport Nutrition, 7*(3), 197-206. Retrieved from http://epubs.scu.edu.au/cgi/viewcontent.cgi?article=1443&context=hahs_pubs

Wilkinson, D. J., Hossain, T., Hill, D. S., Phillips, B. E., Crossland, H., Williams, J., . . . Phillips, S. M. (2013). Effects of leucine and its metabolite β-hydroxy-β-methylbutyrate on human skeletal muscle protein metabolism. *The Journal of Physiology, 591*(11), 2911-2923. doi:10.1113/jphysiol.2013.253203

Wilson, G. J., Wilson, J. M., & Manninen, A. H. (2008). Effects of β-hydroxy-β-methylbutyrate (HMB) on exercise performance and body composition across varying levels of age, sex, and training experience: A review. *Nutrition & Metabolism (London), 5*, 1. doi:10.1186/1743-7075-5-1

Wilson, J. M., Fitschen, P. J., Campbell, B., Wilson, G. J., Zanchi, N., Taylor, L., . . . Hoffman, J. R. (2013). International society of sports nutrition position stand: β-hydroxy-β-methylbutyrate (HMB). *Journal of the International Society of Sports Nutrition, 10*(1), 6. doi:10.1186/1550-2783-10-6

Wilson, J. M., Lowery, R. P., Joy, J. M., Walters, J. A., Baier, S. M., Fuller, J. C., Jr., . . . Wilson, S. (2013). β-Hydroxy-β-methylbutyrate free acid reduces markers of exercise-induced muscle damage and improves recovery in resistance-trained men. *British Journal of Nutrition, 110*(03), 538. doi:10.1017/S0007114512005387

Zuniga, J. M., Bubak, M. P., Fisher, B. E., Neighbors, D. E., Osowski, H. S., & Oyen, A. N. (2013). Electromyographic and gas exchange fatigue thresholds during incremental treadmill running. *Journal of Athletic Medicine, 1*(2), 92-103. Retrieved from http://jampub.com/index.php/JAM/article/viewFile/37/21