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The Effects of High-Intensity Interval Training and 28 days of [Beta]-Hydroxy-[Beta]-Methybutyrate Supplementation on Measures of Aerobic Power and Metabolic Thresholds

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THE EFFECTS OF HIGH-INTENSITY INTERVAL TRAINING AND 28 DAYS OF β-HYDROXY-β-METHYLBUTYRATE SUPPLEMENTATION ON MEASURES OF AEROBIC POWER AND METABOLIC THRESHOLDS

by

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ABSTRACT

Purpose: To examine the effects of 28 days of β-hydroxy-β-methylbutyrate free acid (HMB) and high-intensity interval training (HIIT) on maximal oxygen consumption ($\dot{V}O_2$peak), ventilatory threshold (VT), respiratory compensation point (RCP) and time to exhaustion (T_{max}) in college-aged men and women. **Methods:** Healthy men and women (n=34, age and $\dot{V}O_2$peak= 22.7±3.1yr and 39.3±5.0 mL$^{.}$kg$^{-1.}$min^{-1}, respectively) participated in this study. All participants completed a series of tests prior and subsequent to treatment. A maximal oxygen consumption test was performed on a cycle ergometer to assess $\dot{V}O_2$peak, T_{max}, VT, and RCP. The peak power output (P_{peak}), power at VT (PVT) and power at RCP (PRCP) were also recorded from this test. Twenty-six subjects completed 12 HIIT (80-120% maximal workload) exercise sessions consisting of 5-6 bouts of a 2:1 minute cycling work to rest ratio protocol over a four-week period, while eight served as controls (CTL). In double-blind fashion, the HIIT groups were assigned into either a placebo (HIIT) or 3g per day of HMB (HMB-HIIT). Body composition was measured with dual energy x-ray absorptiometry (DEXA). Outcomes were assessed by ANCOVA with posttest means adjusted for pretest differences. **Results:** The HMB-HIIT intervention showed significant ($p<0.05$) gains in $\dot{V}O_2$peak, VT, and PVT versus the CTL and HIIT group. Both HIIT and HMB-HIIT treatment groups demonstrated significant ($p<0.05$) improvement over CTL for $P_{peak,}$ T_{max}, RCP, and PRCP with no significant difference between the treatment groups. There were no significant differences observed for any measures of body composition. An independent-samples t-test confirmed that there were no significant differences between the training volumes for the HIIT and HMB-HIIT groups. **Conclusion**: These findings suggest that the addition of HMB supplementation may result in greater changes in $\dot{V}O_2$peak and

VT than HIIT alone. Therefore, in college-aged men and women, the use of HMB

supplementation may enhance the benefits of HIIT on aerobic performance measures.

Keywords: High-Intensity Interval Training, β-Hydroxy-β-Methylbutyrate, Aerobic, Endurance,

$\dot{V}O_2$peak, Respiratory Compensation Point, Ventilatory Threshold

ACKNOWLEDGMENTS

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CHAPTER I

High-intensity interval training (HIIT) has become a popular training modality over the past 10 years due to its potential benefits in athletes, untrained individuals, and clinical populations. HIIT consists of repeated bouts of short to moderate duration exercise completed at intensities greater than the anaerobic threshold, interspersed with brief periods of low intensity or passive rest. The salient features of HIIT over constant rate aerobic training (CRT) are shorter training periods and the reported improvements of both the oxidative and glycolytic systems (Laursen & Jenkins, 2002; Perry, Heigenhauser, Bonen, & Spriet, 2008).

HIIT is designed to repeatedly stress the body physiologically, resulting in chronic adaptations and improved metabolic efficiency (Jenkins & Quigley, 1993; Laursen, Shing, Peake, Coombes, & Jenkins, 2005). Specifically, these adaptations include altered skeletal muscle substrate utilization and improved respiratory control sensitivity resulting from increased mitochondrial density (Jacobs et al., 2013). Helgerud et al. (2007) reported that an eight-week running HIIT program improved $\dot{V}O_2$max and TTE more than CRT in moderately trained males. Further, Smith et al. (2009) reported a 9% to 11% increase in $\dot{V}O_2$peak and ventilatory threshold (VT) values after only 3-weeks of HIIT on a cycle ergometer using college age males. HIIT, therefore, is a time-efficient exercise strategy that has shown consistent positive results following relatively short training programs.

The branched-chain amino acid, leucine, has shown to be the key contributor for muscle protein synthesis and may play a role as a substrate during this process (Churchward-Venne et al., 2014). As such, dietary supplementation of leucine and its metabolites have been demonstrated to provide anabolic or anti-catabolic effects on lean body mass during training or periods of energy imbalance (Carbone, McClung, & Pasiakos, 2012; Katsanos, Kobayashi,

Sheffield-Moore, Aarsland, & Wolfe, 2006; Norton & Layman, 2006). Ingestion of one of these metabolites, β-hydroxy-β-methylbutyrate (HMB), has been suggested to provide similar benefits to those of leucine with regard to muscle protein synthesis (Wilkinson et al., 2013). Additional investigation with HMB and resistance training in humans has shown improvement in muscle mass and strength in both younger and older samples (Flakoll et al., 2004; Nissen et al., 1996; J. R. Stout et al., 2013; G. J. Wilson, Wilson, & Manninen, 2008). Recently, scientists have suggested HMB may enhance the benefits of aerobic training. Knitter et al. (2000) proposed that HMB might improve intense aerobic training by attenuating skeletal muscle damage and accelerating recovery between training bouts. In support, Knitter et al. (2000) examined the effect of three grams of HMB or placebo per day in trained endurance athletes for six weeks. Following the training and supplementation period, blood markers of muscle damage, creatine phosphokinase (CPK) and lactate dehydrogenase (LDH), were measured in response to a 20-km race. Immediately and 24-hours post-run, LDH and CPK levels were 10.5% and 17% lower in the HMB supplemented group, respectively. The results from Knitter et al. (2000) suggested that HMB supplementation may attenuate some of the muscle damage often observed with endurance training, possibly reducing the incidence of overtraining and allowing for greater training adaptations.

The physiological mechanisms influenced through HMB supplementation and training may also confer improvements in exercise performance. In support, Vukovich and Dreifort (2001) examined the effect of 3grams of HMB or placebo per day for 14 days in elite cyclists while average training volume was 300 miles per week. In response to only the HMB condition, the cyclists demonstrated a significant increase in peak oxygen consumption rate ($\dot{V}O_2$peak) and an increase in the onset of blood lactate accumulation during a graded exercise test. Vukovich

and Driefort (2001) suggested that changes in maximal and submaximal performance after HMB supplementation may be due to both the attenuation of protein breakdown and the augmentation of mitochondrial protein synthesis resulting in greater oxidative energy capacity.

More recently, Lamboley et al. (2007) examined the effect of 5 weeks of HMB supplementation and HIIT on a treadmill in physically-active college students. Specifically, they measured changes in $\dot{V}O_2$max, VT and respiratory compensation point (RCP) during a graded exercise test at baseline and post training. The 5 weeks of running HIIT was completed 3 times per week on a 1% graded treadmill and participants supplemented 3 grams per day of HMB or placebo. The results of their investigation demonstrated significant increases in $\dot{V}O_2$max, VT and RCP in both treatment groups from the HIIT, however, HMB resulted in a 19% to 45% greater increase in all metabolic variables. The authors suggested that HMB may have attenuated the muscle damage often observed from running and might have accelerated recovery between training bouts. Further, HMB supplementation could have enhanced the training stimulus of HIIT on VT and RCP by increasing mitochondrial biogenesis, thus improving oxidative energy capacity and efficiency (Stancliffe, 2012).

It appears that HMB supplementation is most effective during muscle damaging exercise (J. M. Wilson, Lowery et al., 2013). Lamboley et al. (2007) indicated that they specifically selected running to induce delayed onset muscle soreness, a non-invasive indicator of muscle damage. However, to date, no one has examined the effect of HMB supplementation while undergoing a chronic HIIT program on a cycle ergometer. If muscle damage is needed to observe the potential benefits of HMB supplementation, then HIIT training on a cycle ergometer, which produces much less muscle damage (Koller et al., 1998) than running, may provide no additional benefit. Therefore, the purpose of this study was to examine the effects of chronic (4-

weeks) supplementation in combination with cycle ergometry HIIT on endurance performance measures in active college age men and women.

Hypotheses

1. It was hypothesized that four weeks of HMB supplementation combined with HIIT would significantly increase $\dot{V}O_2$peak, VT, RCP and time to exhaustion in active individuals

2. It was hypothesized that four weeks of HIIT alone would be significantly increase $\dot{V}O_2$peak, VT, RCP and time to exhaustion.

3. It was hypothesized that four weeks of HMB supplementation combined with HIIT would increase $\dot{V}O_2$peak, VT, RCP and time to exhaustion in recreationally trained individuals significantly more than HIIT alone.

4. It was hypothesized that four weeks of HMB supplementation combined with HIIT would alter body composition in active males and females.

Theoretical Assumptions

1. Subjects accurately answered the medical history and activity questionnaire.

2. All subjects gave maximal effort when performing the $\dot{V}O_2$peak 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

Review Of Literature

High-intensity interval training (HIIT) consists of repeated bouts of short to moderate duration exercise completed at an intensity greater than the anaerobic threshold, interspersed with brief periods of low intensity or passive rest. HIIT is designed to repeatedly stress the body physiologically, resulting in chronic adaptations and improving metabolic and energy efficiency significantly faster and greater than traditional constant rate aerobic training (Helgerud et al., 2007; Jenkins & Quigley, 1993; Laursen et al., 2005). Specifically, these adaptations may include a change in skeletal muscle substrate utilization and improved respiratory control sensitivity, resulting from increased mitochondrial density (Jacobs et al., 2013).

A few scientists have suggested that the metabolite of leucine, β-hydroxy-β-methylbutyrate (HMB) may enhance the benefits of aerobic training (Knitter, Panton, Rathmacher, Petersen, & Sharp, 2000; Lamboley, Royer, & Dionne, 2007; Vukovich & Dreifort, 2001). Knitter et al. (2000) proposed that HMB may improve intense aerobic training by attenuating skeletal muscle damage and accelerating recovery between training bouts leading to greater training adaptations. In support, Vukovich and Driefort (2001) examined the effect of 3grams of HMB or placebo per day for 14 days in elite cyclist while average training volume was 300 miles per week. Only during the HMB supplementation period, did the cyclist demonstrate a significant increase in peak oxygen consumption rate ($\dot{V}O_2$peak), and an increase in the onset of blood lactate accumulation during a graded exercise test. Further, Lamboley et al. (2007) examined the effect of 5 weeks of HMB supplementation and HIIT on a treadmill in physically-active college students. Specifically, they measured changes in $\dot{V}O_2$max, VT and respiratory compensation point (RCP) during a graded exercise test at baseline and post training.

The 5 weeks of running HIIT was completed 3 times per week on a 1% graded treadmill and participants supplemented 3 grams per day of HMB or placebo. The results demonstrated significant increases in $\dot{V}O_2$max, VT and RCP in both treatment groups from the HIIT; however, HMB resulted in a 19% to 45% greater increase in all metabolic variables. To date, research suggests that HMB supplementation in conjunction with endurance training may improve maximal and submaximal performance, not only by attenuating protein breakdown, but may be augmenting the mitochondrial protein synthesis for greater oxidative energy capacity (Knitter et al., 2000; Lamboley et al., 2007; Vukovich & Dreifort, 2001).

Effects of High Intensity Interval Training $\dot{V}O_2$peak

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 seven HIIT sessions over 2 week on skeletal muscle fuel content, mitochondrial enzyme activities, fatty acid transport proteins, $\dot{V}O_{2\,peak}$, and whole body metabolic, hormonal, and cardiovascular responses to exercise. Eight female participants performed a $\dot{V}O_{2\,peak}$ test and a 60-min cycling trial at ~60% $\dot{V}O_{2\,peak}$ before and after training.

Each HIIT session consisted of ten 4-min bouts at ~90% $\dot{V}O_{2\,peak}$ with 2 min of rest between intervals. Training increased $\dot{V}O_{2\,peak}$ by 13%. After HIIT, plasma epinephrine and heart rate decreased during the final 30 min of the 60-min cycling trial at ~60% pre-training $\dot{V}O_{2\,peak}$.

Exercise whole body fat oxidation increased by 36%, and net glycogen use was reduced during the post-training 60-min cycling trial. HIIT significantly increased muscle mitochondrial β-hydroxyacyl-CoA dehydrogenase 31.8% and citrate synthase 19.88% 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 wk at a training intensity of ~90% VO2max was sufficient to improve

VO2peak and resulted in increases in whole body and skeletal muscle capacity for fatty acid

oxidation during exercise in moderately active women.

Duffield, Edge, Bishop (2006)
Effects of high-intensity interval training on the VO$_2$ response during severe exercise

The purpose of this study was to examine the effect of high-intensity interval training on the VO$_2$

response during severe, constant-load exercise. Prior to, and following training, 10 females

performed a graded exercise test to determine VO$_2$peak and lactate threshold and a 6 min cycle

test at the pre-training VO$_2$peak intensity. Training involved high-intensity intervals (2 min

work, 1 min rest) performed 3× week for 8 weeks. The training protocol involved a periodized

increase in volume and intensity over the 8-week program, with a taper during the final week to

simulate the style of true athletic competition training. Individual programs were tailored to

participants using a percentage of the lactate threshold measured during the GXT. This training

protocol resulted in significant improvements in VO$_2$peak, 20.87%, power at VO$_2$ max, 20% and

power at LT, 20.35%. In conclusion, a severe-intensity interval-training program significantly

increased VO$_2$ peak and power at both VO$_2$peak and the lactate threshold in active females

without altering the speed of the VO$_2$ response to severe-intensity exercise. The greater VO$_2$

response also decreased the accumulated oxygen deficit, which resulted in a significantly

reduced reliance on anaerobic metabolism at the same absolute intensity. Therefore, training at

high intensities can increase oxygen consumption at maximal exercise intensities without

slowing the speed of the aerobic response.

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

Aerobic High-Intensity Intervals Improve $\dot{V}O_2$max 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, VO_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 VO_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 VO_2max from 60.5 to 64.4 mL·kg^{-1}·min^{-1} and 55.5 to 60.4 mL·kg^{-1}·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 VO_2max. The authors also state that improvements in VO_2max seem to be dependent on initial fitness level and type of exercise normally performed by the individual; individuals who performed short, intense burst-style, similar to football, saw no change in VO_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.

Smith, Walter, Graef, Kendall, Moon, Lockwood, Fukuda, Beck, Cramer, Stout (2009)
Effects of β-alaninesupplementation and high-intensity interval training on endurance performance and body composition in men; a double-blind trial

The focus of this study was to evaluate the effects of combining β-alanine supplementation with HIIT on endurance performance and aerobic metabolism in recreationally active college-aged men. Forty-six men were tested for VO_2peak, time to fatigue, ventilatory threshold, and total work done at 110% of pre-training VO_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 training 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 VO_2peak test and progressed in an undulating manner, reaching a maximum of 115% of peak power. Significant improvements in VO_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 induces 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 main aim of this study was to examine the effects of combining sodium bicarbonate supplementation with HIIT on endurance performance and aerobic metabolism. Thirty-six recreationally active college aged men gave their informed consent and volunteer to participate

in the study. VO_2peak, time to fatigue, ventilatory threshold, and total work done at 110% of

pre-training VO2peak were assessed. In double-blind conditions, all subjects were randomly

assigned into one 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 training

consisting of 5-6 bouts of a 2:1 minute cycling work to rest ratio. The results suggest that the

significant improvements in VO_2peak, time to exhaustion, and total work after three weeks of

training were observed. The findings highlight the use of HIIT to induce significant aerobic

improvements is effective and efficient. Chronic sodium bicarbonate supplementation may

further enhance HIIT, improving endurance performance and lean body mass.

A Review of Respiratory and Metabolic Thresholds During Exercise

Beaver, Waserman, Whipp (1986)
A new method for detecting anaerobic threshold

The purpose of this study was to examine the relationship between VO_2 and CO_2 versus the VE

to determine how each reacts to a graded exercise test and to relate those responses to

physiological responses within the body. Excess CO_2 is generated when lactate is increased

during exercise because it $[H^+]$ is buffered primarily by bicarbonate. The researchers have

developed a method to detect the anaerobic threshold, using computerized regression analysis of

the slopes of the CO_2 uptake (VCO_2) vs. O_2 uptake (VO_2) plot, which detects the beginning of

the excess CO_2 output generated from the buffering of H+ ions, termed the V-slope method.

From incremental exercise tests on 10 subjects, the point of excess CO_2 output closely predicted

the lactate and bicarbonate thresholds. The mean gas exchange anaerobic threshold was found to

correspond to a small increment of lactate above the mathematically defined lactate threshold

[0.50 +/- 0.34 (SD) meq/l] and not to differ significantly from the estimated HCO^{-3} threshold.

The mean VO_2 at anaerobic threshold computed by the V-slope analysis did not differ significantly from the mean value determined by a panel of six experienced reviewers using traditional visual methods, but the anaerobic threshold could be more reliably determined by the V-slope method. The respiratory compensation point, detected separately by utilizing the V-slope method to examine the minute VE vs. VCO_2 plot, was consistently higher than the anaerobic threshold (2.51 +/- 0.42 vs. 1.83 +/- 0.30 l/min of VO_2). This V-slope method for determining the anaerobic threshold and respiratory compensation point may detect the increased CO_2 production from buffering metabolic acid and may provide a more widely accepted and applicable method for estimating metabolic thresholds.

Caiozzo, Davis, Ellie, Azus, Vandagriff, Prietto, McMaster (1982)
A comparison of gas exchange indices used to detect the anaerobic threshold

This study was designed to examine four commonly used ventilatory or gas exchange indices and determine which may provide the most accurate and reliable detection of the anaerobic threshold. Sixteen participants performed two graded exercise tests on a cycle ergometer to volitional fatigue. After 4 min of unloaded cycling, the work rate was increased 20 W/min. Ventilatory and gas exchange measurements were made every 30 sec throughout each test. During one of the two tests, venous blood was also sampled every 30 sec for subsequent determinations of blood lactate concentration. VE, VCO_2, RER, VE/VO_2 were used separately to detect the anaerobic threshold. The anaerobic threshold determined from systematic increases in lactate concentration was used as the criterion measure. Anaerobic threshold values were found using VE(1.79 +/- 0.11), VCO_2(1.74 +/- 0.11), RER(1.58 +/- 0.06), VE/VO_2(1.84 +/- 0.11), and blood lactate (1.85 +/- 0.11 l/min)concentrations. The highest correlation between a ventilatory or gas exchange anaerobic threshold and onset of blood lactate was found to be VE/VO_2 (r=0.93, P < 0.001). The VE/VO_2 was also found to have the highest test-retest

correlation for detection of the anaerobic threshold (r = 0.93, P < 0.001). Multiple correlational

analyses did not significantly enhance detection of the anaerobic threshold. The authors

identified five factors that favor using VE/VO$_2$ to detect the anaerobic threshold: 1) it provides

the highest correlation with onset of blood lactate accumulation; 2) it resulted in the highest test-

retest correlation; 3) VE/VO$_2$ is easily derived from standard ventilatory and gas exchange

measures; 4) VE/VO$_2$ exhibits a tri-phasic pattern that qualitatively allows the investigator to

have more confidence in the determination of anaerobic threshold; and 5) the dual criterion

utilizing VE/VCO$_2$ provides and more specific detection of the anaerobic threshold. Therefore,

the authors believe that the use of VE/VO$_2$ for noninvasive detection of the anaerobic threshold

produces the most sensitive and reliable ventilatory or gas exchange index studied.

Meyer, Faude, Scharhag, Urhausen, Kindermann (2003)
Is lactic acidosis a cause of exercise induced hyperventilation at the respiratory compensation point?

The aim of this study was to discover the role of pH changes in the onset of hyperventilation play

in incremental exercise. Five healthy participants completed a first ramp-like exercise test on a

cycle ergometer, and respiratory compensation point was determined from gas exchange

measurements in subjects. The first exercise served as a baseline test to determine the course

and degree of the pH decline and the normal ventilatory stress reaction. In the second test,

sodium bicarbonate was injected intravenously in small doses during a second, otherwise

identical, exercise test to offset blood acidification. In each subject sufficient compensation for

the acidosis, that is, a pH value constantly above 7.37, was attained during the second test. A

delay but no disappearance of the hyperventilation was present in all participants when compared

with the first test. The respiratory compensation point occurred on average at a significantly

(p=0.043) higher oxygen uptake (+0.15 l*min^{-1}) compared with the first test. This study

demonstrates that the respiratory compensation point was delayed when exercise induced blood acidosis was prevented by intravenously injecting bicarbonate. This experiment provides evidence that changes in blood pH are involved in the initiation of the respiratory compensation point. However, other physiological stimuli for hyperventilation seem to be present. Therefore, it is concluded that exercise induced lactic acidosis is causally involved in the hyperventilation, which starts at the respiratory compensation point. However, the authors cautioned that lactic acidosis does not represent the only additional stimulus of ventilation during intense exercise.

Paterson, Friedland, Bascom, Clement, Cunningham, Painter, Robbins (1990)
Changes in arterial K+ and ventilation during exercise in normal subjects and subjects with McArdle's syndrome

The goal of this study was to examine the relationship between VE, lactate and arterial plasma K^+ concentrations during incremental exercise in six normal subjects and in four subjects with McArdle's syndrome who do not become acidotic during exercise. During the exercise test the work rate was increased each minute by increments of 25W for the normal participants and 5W for the participants with McArdle's syndrome until both groups reached the point of volitional fatigue. This point was attained when a rating of perceived exertion of 19 was reached on the Borg scale or when a pedal frequency of 60 rpm could no longer be maintained. Blood for potassium analysis was sampled from the catheter into heparinized syringes at the end of each minute of exercise and during the 10 min recovery period. In normal subjects, arterial plasma K^+ rose to *ca*7 m**M** at the point of exhaustion. The time courses of the increases in VE, lactate and arterial plasma K^+ were all similar during the exercise period. Lactate reached its peak concentration during the recovery from exercise when both VE and arterial plasma K+ were returning to baseline levels. For the participants with McArdle's syndrome a non-linear ventilatory response during incremental exercise was observed. Arterial plasma K^+ was in this

group was closely related to VE throughout exercise and recovery. Arterial pH in the McArdle's syndrome participants increased from the onset of exercise, rather than remaining constant. At all stages of exercise, the levels of VE and arterial plasma K^+ concentrations were greater in the participants with McArdle's syndrome than in the control group. The unique finding of this study was that that the close relationship of hyperkalemia and VE during a graded exercise test may contribute significantly to the drive to breathe—in both a normal population and in individuals with McArdle's syndrome for whom acid plays no role in ventilation during exercise—and may stimulate hyperventilation during heavy exercise.

Effect of Training on Respiratory Compensation Point and Ventilatory Threshold

Oshima, Tanaka, Miyamoto, Wadazumi, Kurihura, Fujimoto (1998)
Effects of endurance training above the anaerobic threshold on isocapnic buffering phase during incremental exercise in middle-distance runners

A study was performed to clarify the effects of endurance training above the anaerobic threshold on the isocapnic buffering phase during incremental exercise in athletes. Eight middle-distance runners performed a graded exercise test utilizing a modified version of the Bruce protocol. After 6-months of HIIT and paced running training at levels above anaerobic threshold, VO_2max was significantly different from 60.1±5.7 to 64.7±5.5, a 7.65% increase. Anaerobic threshold increased 4.9%, which although slight, showed a significant increase from pre-training. The respiratory compensation point also saw significant and marked increase of 7.2% above the baseline testing value. Although neither the slope of the first regression line below anaerobic threshold nor that of the second line above anaerobic threshold calculated by V-slope analysis was altered, the range of isocapnic buffering from anaerobic threshold to respiratory compensation point was significantly extended from 24.8±5.9 to 28.1±6.0 after the 6-months of training. In addition, the amount of change in VO_2max after the 6-month training period was

correlated with the change in isocapnic buffering (R=0.72, p<0.05). One drawback to the study was that training was unstructured, individuals were allowed to train on their own with the instruction to ensure that during interval training, sprints were performed in an "all out" effort. The authors concluded that the degree of increased respiratory compensation point is larger than that of anaerobic threshold after high-intensity endurance training at levels above anaerobic threshold and that the range of isocapnic buffering may be an important factor in relation to the increase in the maximal aerobic capacity of athletes.

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 vs. HIIT training 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 wk of detraining. $\dot{V}O_2$max 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 (Qmax, from 18.1 ± 1.1 to 20.1 ± 1.2 l/min; $P < 0.01$). Only HIIT training 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 $\dot{V}O_2$max with HIIT. The gain of Qmax was also correlated with the gain of VO$_2$max. 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 type over continuous-type training on endurance performance enhancement. Moreover, our observations suggest that enhancements of muscular mitochondrial function are actively involved in the observed VO_2max improvements. Therefore, HIIT seems optimal in maximizing both peripheral muscle and central cardiorespiratory adaptations, permitting significant functional improvement.

Effect of High-Intensity Interval Training on Body Composition

Tremblay, Simoneau, Bouchard (1994)
Impact of exercise intensity on body fatness and skeletal muscle metabolism

The present study was aimed at re-evaluating the hypothesis—that for a given level of energy expenditure, individuals engaging in vigorous activities are leaner than those participating in less intense activities and inactive people—by comparing the impact on body fatness of a program including only aerobic exercise of moderate intensity versus a high-intensity exercise program. The current study attempted to provide a mechanistic explanation for the potential difference in fat loss between the two exercise programs. Therefore, they examined the impact of two different modes of training on body fatness and skeletal muscle metabolism in young adults. Twenty-seven men and women were assigned to either a 20-week continuous endurance training program (8 men; 9 women) or a 15-week HIIT program (5 men; 5 women). The HIIT group performed 25 30-minute sessions of continuous exercise at 70% of the maximal heart rate reserve, which approximately corresponded to an intensity comparable to the intensity of the individuals exercising in the continuous endurance training program. In addition, they performed 19 short- and 16 long-interval sessions over a period of 1.5weeks. The mean

estimated total energy cost of the continuous endurance training program was 120.4 MJ, whereas

the corresponding value for the HIIT program was 57.9 MJ. Despite its lower energy cost, the

HIIT program induced a more pronounced reduction in subcutaneous adiposity compared with

the continuous endurance training program. There was no significant change in body weight in

response to either the continuous endurance training or the HIIT program. Both programs

induced significant reductions in the suprailiac skinfold and the sum of three trunk subcutaneous

skinfolds. In addition, a significant decrease in the triceps, biceps, and subscapular skinfolds as

well as the sum of three limb skinfolds and the sum of the six skinfolds, was observed following

the HIIT program. When corrected for the energy cost of training, the decrease in the sum of six

subcutaneous skinfolds induced by the HIIT program was nine fold greater than by the

continuous endurance training program. Muscle biopsies obtained in the vastus lateralis before

and after training showed that both training programs similarly increased the level of the citric

acid cycle enzymatic marker. On the other hand, the HIIT program increased the activity of

muscle glycolytic enzymes, whereas a decrease was observed following the continuous

endurance training program. The enhancing effect of training on muscle 3-hydroxyacyl

coenzyme A dehydrogenase (HADH) enzyme activity, a marker of the activity of β-oxidation,

was significantly greater after the HIIT program. Although unable to explain the effect of

exercise intensity on energy balance, the authors speculate that following a vigorous exercise,

energy intake is either transitorily suppressed or increased to a lesser degree compared with that

following an exercise of low to moderate intensity. Additionally, it is also possible that vigorous

exercise has a greater enhancing effect on post-exercise energy expenditure than that of moderate

exercise. These results reinforce the notion that for a given level of energy expenditure, vigorous

exercise favors negative energy and lipid balance to a greater extent than exercise of low to

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moderate intensity. Moreover, the metabolic adaptations taking place in the skeletal muscle in response to the HIIT program appear to favor the process of lipid oxidation.

Keating, Machan, O'Connor, Gerofi, Sainsbury, Caterson, Johnson (2014)
Continuous Exercise but Not High Intensity Interval Training Improves Fat Distribution in Overweight Adults

The purpose of this study was to assess by randomized placebo-controlled trial the effect of 12 weeks of HIIT versus continuous aerobic exercise versus a sham-exercise placebo control on body composition and cardiovascular risk factors in overweight, previously inactive adults. Work capacity and body composition, via DEXA, were measured before and after 12 weeks of intervention in 38 previously inactive overweight adults. For the exercise interventions, the HIIT program consisted of repeated bursts of exercise on the cycle ergometer at a power output designed to elicit 120% of VO_2peak. Each exercise bout was interspersed with an active rest period of cycling at a low intensity of 30 W. Intervals were progressed over the first four weeks from 4 intervals on a work: recovery schedule of 30–45 sec at 120% of VO_2peak: 2–3 min active recovery. For weeks 5–12 participants performed 6 intervals on a work: recovery schedule of 60 sec exercise: 120 sec active recovery. The continuous endurance training program involved continuous cycling on an ergometer. Training was began with 30 minutes at an intensity of 50% of VO_2peak in week one and progressively increased to 45 minutes at an intensity of 65% of VO_2peak by week 5 of the study. There was a significant group × time interaction for change in work capacity, which increased significantly in the continuous endurance training by 23.8% and in the HIIT group by 22.3% but not placebo group, which saw an increase of 3.1%. While there was no significant main effect for percentage trunk fat, trunk fat reduced in the continuous endurance training by 3.1% and in PLA by 1.1%, but not in HIIT, which increased 0.7%. There was also a significant reduction in android fat percentage in continuous endurance training of

2.7% and in the placebo group PLA of 1.4%, but again, not in the HIIT group where an increase

of 0.8% was observed. Due to the fact that this study observed no significant differences in

dietary intake and non-exercise physical activity, the changes in body composition are arguably

reflective of the effect of the interventions. These data suggest that HIIT may be advocated as a

time-efficient strategy for eliciting comparable fitness benefits to traditional continuous exercise

in inactive, overweight adults. However, in this population HIIT does not confer the same

benefit to body fat levels as continuous exercise training.

<center>β-Hydroxy-β-Methylbutyrate Metabolism and Mechanisms</center>

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 objective of this work was to present the collective safety data from nine studies in which

humans were fed 3 g HMB/d. The studies were from 3 to 8 wk in duration, the sample

populations of the studies included both males and females, young and old individuals, and

exercising or non-exercising populations. Organ and tissue function was assessed by blood

chemistry and hematology; subtle effects on emotional perception were measured with 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, which translates into 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

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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. Therefore, the

authors advise that HMB can be taken safely as an ergogenic aid for exercise and that the use of

supplementing 3 g/d of HMB as an ergogenic aid for exercise is well tolerated and safe in

humans.

Holecek, Muthny, Kovarik, Sispera (2009)
Effect of beta-hydroxy-beta-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 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 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
Effects of leucine and its metabolite β-hydroxy-β-methylbutyrate on human skeletal muscle protein metabolism

This study aimed to investigate the possibility that HMB could represent an anabolic metabolite of leucine by studying the effects of HMB on human muscle protein turnover and compared with that of leucine. The authors carried the following three hypotheses into the experiment: 1) HMB provision would acutely stimulate MPS; 2) HMB would stimulate muscle protein synthesis through mechanisms similar to its precursor, leucine; 3) HMB 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 study 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 portion of the study [^{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 free-acid HMB 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 arterio-venous

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 by110%). While HMB 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 ($\sim$56%) and leucine ($\sim$45%) groups at 30min. However, by

the 90 min time point increased p70S6K1 phosphorylation was maintained ($\sim$71%) only in the

leucine group. HMB 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 induces acute muscle

anabolism via increased muscle protein synthesis and reduced muscle protein breakdown

although there is still uncertainty if HMB acts separately or in conjunction with the

mechanism(s) of leucine.

Pinheiro, Gerlinger-Romero, es-Ferreira, de Souza-Jr, Vitzel, Nachbar, Nunes, Curi (2011)
**Metabolic and functional effects of beta-hydroxy-beta- methylbutyrate (HMB)
supplementation in skeletal muscle**

The aim of this study was to evaluate the effects of HMB supplementation for 4 weeks upon

metabolic parameters and skeletal muscle contractile function in rats. In the present study, the

effects of HMB supplementation on ATP and glycogen content, citrate synthase activity, maximum strength production, resistance to acute fatigue, contraction velocity and relaxation capacity in skeletal muscle were investigated. Wistar rats were supplemented with 320 mg/kg of body weight per day HMB for 4 weeks. A placebo group received the same volume of a saline only 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 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-supplemented rats compared to placebo group. Muscle tetanic force was increased by HMB supplementation. 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 supplementation. Glycogen content was increased fivefold in white and fourfold in red portions of gastrocnemius muscle. HMB 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 proposition that HMB supplementation results in metabolic changes that are associated with increased muscle strength generation and prevention of acute muscle fatigue via marked change in oxidative metabolism during intense contractions.

Kimura, Cheng, Inoue, Hu, Koike, Kuzuya (2014)

β-Hydroxy-β-methylbutyrate facilitates PI3K/Akt-dependent mTOR and FoxO1/3a phosphorylations and alleviates TNF-α/IFN-γ-induced MuRF-1 expression in C2C12 cells

In this study, researchers investigated the hypothesis that HMB may modulate the balance between protein synthesis and degradation in the PI3K/Akt-mediated mammalian target of rapamycin (mTOR) and FOXO1/FOXO3a-dependent mechanisms in differentiated C2C12 muscle cells. This study also tested the effect of HMB on the expression of MuRF-1 and atrogin-1 in response to the inflammatory stress. C2C12 cells were cultured in serum-free DMEM for 12 h, the cells were treated with 50 μM of HMB at time points: 0, 10, 30, and 60 min and 24 h, for the Western blotting assay of targeted protein phosphorylations. Quantitative gene expression was studied by using an ABI 7300 Real-Time PCR System. The protein concentration for each sample was determined using a protein assay system and protein bands were developed by the chemiluminescence method and detected using a digital imaging system. HMB upregulated phosphorylation of Akt and mTOR beginning at the 10 min sample and reached a 300% peak at 30 min, but had declined by the 60 min sampling point after the intervention. Additionally, these effects were completely abolished in the presence of PI3K inhibitor LY294002. Cells stimulated with HMB at 50 μM for 30 min upregulated FOXO1 and FOXO3a phosphorylation. These changes were inhibited by LY294002, indicating that the HMB-mediated FOXO1 and FOXO3a phosphorylations may be due to PI3K/Akt signaling pathway activation. Kimura et al demonstrated that HMB failed to reduce the expressions of atrophy-related atrogin-1 mRNA and the protein response to the pro-inflammatory cytokines tumor Necrosis Factor-α (TNF-α) plus interferon-γ (IFN-γ). However, HMB did attenuate the expression of the MuRF-1 gene. With respect to the effect of HMB on the prevention muscle atrophy, Kimura and colleagues intimate that HMB appears to restore the balance between

intracellular protein synthesis and proteolysis, likely via activation of the PI3K/Akt-dependent

mTOR and FoxO1/FoxO3 signaling pathway and the reduction of TNF-α/IFN-γ-induced MuRF-

1 expression. The findings of the present study suggest that HMB supplementation may be

beneficial for countering aging-related muscle wasting and muscle atrophy helping to limit

imbalance between protein synthesis and ubiquitin-proteasome-mediated proteolysis.

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 could improve

HMB bioavailability to tissues over the previous Calcium salt form of HMB. 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 CaHMB 2) equivalent HMB free acid gel swallowed

and 3) free acid gel 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

calcium salt form of HMB, 38 min for HMB swallowed immediately and 38 min for HMB held

sublingually. Similarly, the authors also found a significant difference in the peak concentrations

between the different forms of HMB: 131 μmol/l for the calcium salt HMB, 249 μmol/l HMB

swallowed immediately and 239 μmol/l for HMB 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: 3.17hr for the calcium salt HMB , 2.50hr for HMB

swallowed and 2.51hr for HMB 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 calcium salt form of HMB administered in a capsule, is equally

safe and may improve HMB availability and efficacy to tissues in health and disease.

<p align="center">Effect of β-Hydroxy-β-Methylbutyrate Free Acid on Recovery</p>

Wilson, Lowery, Joy, Walters, Baier, Fuller Jr, Stout, Norton, Sikorski, Wilson, Duncan, Zanchi, Rathmacher (2012)
β-hydroxy-β-methylbutyrate free acid reduces markers of exercise-induced muscle damage and improves recovery in resistance-trained men

The present study was designed to determine the effects of short-term supplementation with the

free acid form of HMB supplement administered just before a bout of resistance exercise on

indices of muscle damage, protein breakdown, recovery and hormone status resistance-trained

athletes. Twenty resistance-trained males were recruited to participate in a high-volume

resistance training session of three sets of twelve maximal repetitions intensity, with a supervised

and timed rest period length of 1min between the sets. The workout consisted of three sets of

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 or a placebo to be consumed divided equally in three servings: 30min 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 assayed via ELISA kits. Serum creatine kinase was measured using colorimetric procedures at 340 nm. The results showed that creatine kinase increased 329% in the placebo group, while the HMB 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 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 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 supplement given to trained individuals prior to intense exercise can blunt increases in muscle damage and may improve perceived readiness for subsequent trainings following a high-volume, muscle-damaging resistance-training session.

Effect 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. The authors hypotheses were that HMB 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 (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 from 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 (0.5 g/kg BW/d)] and CH (= C + HMB 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, C group, had 17% greater lean body mass than the HMB supplemented 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 HMB, 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 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 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 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/wk 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 (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 every day for 4 days after the prolonged run. Serum samples were analyzed for creatine phosphokinase and lactate dehydrogenase activity by a commercial laboratory. Plasma was collected and analyzed for HMB by gas chromatography-mass spectrometry. The placebo-supplemented group exhibited a significantly greater increase in creatine phosphokinase activity in the 4-day period after prolonged run than did the HMB-supplemented 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 with HMB supplementation compared with the placebo-supplemented group. In conclusion, the authors suggest that the fact that the placebo-supplemented subjects exhibited higher lactate dehydrogenase activity after the prolonged run compared with the HMB-supplemented subjects suggests that they would tend to sustain more muscle damage as a result of the run. This fact combined with the lower Creatine phosphokinase levels of the HMB

supplementing group support the authors' hypothesis that HMB supplementation helps prevent exercise-induced muscle damage.

Vukovich, Dreifort (2001)
Effect of [beta]-Hydroxy [beta]-Methylbutyrate on the Onset of Blood Lactate Accumulation and Vo2peak in Endurance-Trained Cyclists

The purposes of this study were to: 1) investigate the effects of HMB supplementation on the onset of blood lactate accumulation and VO_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, leucine, or placebo) each followed by a 2-week washout period. The study utilized cross-over design, so subjects acted as their own controls. For the VO_2peak and time to reach VO_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 VO_2peak. The supplementation protocol consisted of 3 g of cornstarch$\cdot$day^{-1} (CON), 3 g of Ca-HMB$\cdot$ day^{-1}, and 3 g of leucine$\cdot$ 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 VO$_2$peak and time to reach VO$_2$peak resulted in a

significant increase of 4% in VO$_2$peak and 3.5% in time to reach VO$_2$peak 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 VO$_2$ 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 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 prevents protein breakdown and increases protein synthesis, then

cellular and mitochondrial proteins may increase. This may result in a greater 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 beta-hydroxy-beta-methylbutyrate on aerobic-performance components and body composition in college students

The aim of this study was to determine the effects of the calcium salt form of HMB

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 or a

placebo group for a 5-wk supplementation period during which they underwent interval training

3 times a week on a treadmill. 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 VO_2max, as well as ventilatory threshold and respiratory compensation point. Ventilatory threshold was determined to be the intensity corresponding to an increase in $V_E:VO_2$ without a simultaneous increase of $V_E:VCO_2$, the first sustained rise in excess CO_2, and the first increase in the slope of VCO_2 versus VO_2. The authors measured the respiratory compensation point as the intensity corresponding to an increase in both $V_E:VO_2$ and $V_E:VCO_2$, the second sustained rise in excess CO_2, and the second increase in the slope of VCO_2 versus VO_2. Participants were next tested to measure the time to exhaustion at the maximal aerobic speed—defined as the lowest speed that elicited VO_2max during the graded exercise test. Training consisted of a 5-wk training program utilizing interval training on a treadmill 3 times a week. 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 an individual workout, an individual began with a warm-up period of 5 min at 50% of the maximal aerobic speed. After this warm-up, an initial active recovery of 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 the same duration: 50% of the total time to exhaustion. VO_2max for both groups significantly increased after the exercise intervention. However, when the investigators expressed this as a percentage of increase, it indicated that VO_2max increased significantly more in the HMB 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 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 for both of the interventions. However, as with the VO$_2$max, when expressed as a percentage of VO$_2$max, respiratory compensation point significantly improved 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 VO$_2$max and in respiratory compensation point in active subjects thereby positively affecting selected components of aerobic performance in active college students.

CHAPTER III

Participants

For inclusion in the study, all males were required to have a $\dot{V}O_2$peak greater than 35

ml·kg^{-1}·min^{-1} and all female participants greater than 30 ml·kg^{-1}·min^{-1}. After initial testing, forty

recreationally active individuals (Men = 21, Women = 19) between the ages of 18 and 35 were

recruited to participate in this study. Three female and two male participants were removed due

to health reasons not associated with the study. One female participant was removed after a

family emergency. Therefore, data for 19 men and 15 women (Table 1) were included in the

final analysis. All participants completed a questionnaire to assess ability to participant in

physical activity and to ascertain any prior supplementation regime. Individuals were self-

reported to be free of musculoskeletal injury as determined by a PAR-Q. All persons

participating in this study were provided an informed consent to sign and the New England

Institutional Review Board approved the study protocol.

Table 1. Participant Descriptive Data (*N*=34)

Variable	Control (n=8: M=5;F=3)	HIIT (n=13:M=8; F=5)	HMB-HIIT (n=13;M=7; F=6)	F	*p*
Age (y)	21±2.39	23.62±3.73	22.85±2.44	1.907	0.166
Height (cm)	171.43±5.72	172.57±12.21	173±9.22	0.063	0.939
Body Mass (kg)	76.29±12.76	74.92±16.59	72.38±9.87	0.234	0.793
% Body Fat	22.36±8.12	19.68±8.64	24.83±8.09	1.250	0.301
Training Volume (kJ)	N/A	1436.98±309.6	1456.75±378.55	1.06	0.313

A minimum sample size of n=8 per group was determined using previously published

data (Lamboley et al., 2007) and the formula derived by Gravettier and Wallnau (1996) to

achieve a statistical power (1-β) of 0.80. Therefore, with an expectation of subject dropout, a

final sample size of n=15 in each experimental group and n= 10 in the control group were recruited. The study was registered on ClinicalTrials.gov (ID NCT01941368).

Research Design

A double blind, placebo controlled design, stratified for gender, was used to examine the effects of HMB and HIIT training on measures of metabolic performance. Each participant was required to visit the Human Performance Laboratory four times for pre- and post- testing, with each testing session occurring on nonconsecutive days. The same testing protocols were repeated at the end of the 4-week training period. On the first testing day, anthropometric measures of participants were collected (Table 1). Each participant then performed a graded exercise test to determine peak oxygen consumption ($\dot{V}O_2$peak), time to exhaustion (T_{max}), respiratory compensation point (RCP), and ventilatory threshold (VT). The peak wattage achieved during this testwas used to establish individual training intensity. On the second day of testing, a baseline blood draw was performed to measure serum HMB, and total lean soft tissue (TLST) and body fat percentage (BF) were assessed using dual energy x-ray absorptiometry (DEXA) (ProdigyTM; Lunar Corporation, Madison, WI, USA).

After baseline testing, the participants were randomly assigned to one of three groups: a control group (CTL), a placebo+HIIT group (HIIT) or HMB+HIIT group (HMB-HIIT). Of the 40 subjects that were recruited for this study, 10 subjects were assigned to CTL and 15 to each of the training groups (HIIT or HMB-HIIT). In addition, participants were stratified for gender for each group.

Exercise Protocol

Participants in the HIIT and HMB-HIIT groups participated in 4-weeks of high-intensity interval training with three sessions per week—with at least one day between each training session—on

calibrated, electronically-braked cycle ergometer (Corival 400, Groningen, the Netherlands). The exercise training regime consisted of alternating training sessions of sub maximal and supra maximal workloads (Figure 1). Each participant's training load was determined as a percentage of the peak power output (P_{peak}) from the graded exercise test. Individuals began each training session with a 5-minute warm up at a self-selected wattage, followed by a protocol of five 2-minute exercise bouts at a predetermined percentage of their power output at $\dot{V}O_2$peak. Between each exercise bout, the participant had 1 minute of complete rest. Persons assigned to CTL were asked to continue their normal activity pattern for 4 weeks before returning to undergo post-testing.
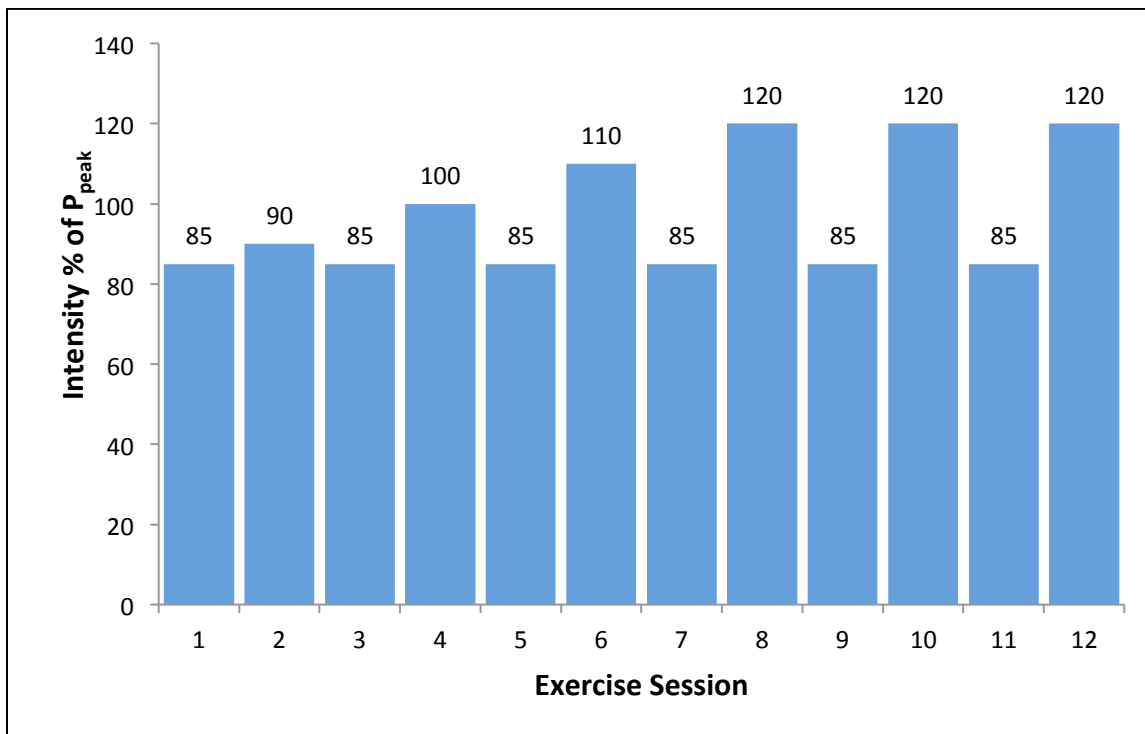
Figure 1. Exercise training intensity protocol

Supplementation

The HMB supplement consisted of 1 gram of β-hydroxy-β-methylbutyrate free acid, reverse osmosis water, de-bittering agent, orange flavor, stevia extract, and potassium carbonate. Each serving of placebo contained 1 gram of polydextrose that was equivalent to β-hydroxy-β-methylbutyrate free acid, citric acid, corn syrup, 10% stevia powder, de-bittering agent, and orange flavoring. The HMB and the placebo were obtained from Metabolic Technologies Inc. (Ames, IA). Prior to the exercise session, subjects were randomly assigned to receive either 3 g per day of HMB or a placebo divided equally into three servings given 30 minutes prior to exercise and again 1 hour later and then final 1g dose 3 hours post exercise. To ensure compliance, investigators watched as the subjects consumed 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 et al. 1990.

Dietary Analysis

Prior to training, participants were asked to complete a 3-day food log, to establish macronutrient content and average leucine intake. 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 calculation for daily leucine intake (g) and total calories (kcal).

Metabolic and Performance Measures

The independent variables included: (a) supplementation treatment [HMB vs. placebo], (b) exercise treatment [HIIT training vs. control]. The dependent variables included: (a) $\dot{V}O_2peak$, (b) T_{max}, (c) RCP, (d) VT, (e) TLST, and (f) BF.

Instrumentation

- An electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) was utilized to perform all graded exercise tests.

- An electronically-braked cycle ergometer (Corival 400, Groningen, The Netherlands) was used for training sessions.

- Open circuit spirometry (True One Metabolic Cart, Parvo Medics, Inc., Sandy UT) was used in the determination of all metabolic measures.

- DEXA (GE Medical Systems Lunar, Madison, WI, USA; software version 13.60.033) was used to determine body composition.

Determination of $\dot{V}O_2peak$, VT, and RCP

An incremental test to volitional exhaustion was performed on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) to determine $\dot{V}O_2peak$ and the peak power output (P_{peak}) in watts (W) at $\dot{V}O_2peak$. Prior to testing, each participant was fitted with a Polar Heart Watch system to record his or her heart rate (Polar Electro Inc., Lake Success, NY). Following the procedures described by Bergstrom et al. (2013), participants were instructed to maintain a pedaling cadence of 70-75 revolutions per minute (RPM) at an initial workload of 75 W. The workload increased 25 W every two minutes until he or she was unable to maintain a cadence above 70 RPM for ~10s despite verbal encouragement, or volitional

fatigue. Prior to each graded exercise test, open-circuit spirometry (True One Metabolic Cart, Parvo Medics, Inc., Sandy UT) was calibrated with room air and gases of known concentration, which was used to estimate $\dot{V}O_2$peak (ml·kg^{-1}·min^{-1}) by sampling and analyzing the breath-by-breath expired gases. Respiratory gases—oxygen (O_2), carbon dioxide (CO_2), ventilation ($\dot{V}_E$), and respiratory exchange ratio (RER)—were monitored continuously and expressed as 30-second averages (Day et al., 2003). $\dot{V}O_2$peak was determined to be the highest 30-s $\dot{V}O_2$ value during the test and coincided with at least two of the following three criteria: (a) 90% of age-predicted maximum heart rate; (b) respiratory exchange ratio > 1.1; and/or (c) a plateau of oxygen uptake (less than 150mL/min increase in $\dot{V}O2$ during the last 60 s of the test). The test-retest reliability for $\dot{V}O_2$peak was ICC= 0.86 (SEM 2.2 ml$^.$kg$^.$min^{-1}).

Ventilatory threshold (VT), and respiratory compensation point (RCP), were determined by common methods for determining gas exchange thresholds (Beaver et al. 1986, Gaskill et al. 2001, Wasserman et al. 1973, Caiozzo et al. 1982) Ventilatory threshold (VT), was determined by plotting and determining the point of increase in the $\dot{V}_E/\dot{V}O_2$ versus $\dot{V}O_2$ curve as the $\dot{V}_E/\dot{V}CO_2$ versus $\dot{V}O_2$ curve remained constant or decreased (Bergstrom et al. 2013, Beaver, Wasserman & Whipp, 1986). The respiratory compensation point (RCP), as described by Beaver et al. (1986), was identified using the V-Slope method by plotting the $\dot{V}_E$ versus $\dot{V}CO_2$. The VT and RCP were reported as the corresponding $\dot{V}O2$ and power output in watts (pVT and pRCP). The test-retest reliability for VT and PVT was ICC= 0.86 (SEM 3.6 ml$^.$kg$^.$min^{-1}) and 0.77 (SEM 16.1 watts), respectively.

Anthropometric Measures

Body composition was estimated from a scan by DEXA (GE Medical Systems Lunar, Madison, WI, USA; software version 13.60.033) performed by a state licensed x-ray technician. Participants were positioned supine in the center of the platform and were scanned using the default scan mode for total body scanning. Measures for lean soft tissue and fat mass for limb, regional, and total body were calculated by the system software (Encore 2011, software version 13.60.033). Body composition was analyzed using estimated body fat percentage (BF) and total body lean mass (LM). The test-retest reliability for BF was ICC= 0.99 (SEM 0.8 %Fat).

Statistical Analysis

Statistical software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp) was used to perform all statistical analysis. Separate 1-way analyses of covariance (ANCOVA) were used to analyze all dependent variable data based on the recommendations of Huck and McLean (1975). The independent variable, group, included 3 levels: HIIT, HMB-HIIT, and CTL. The pretest and posttest values were used as the covariate and dependent variable, respectively. Preliminary least squares regression analyses were conducted to examine the linearity of the relationships between the covariate and the dependent variable within all groups, and the interaction between the covariate and group was used to test for homogeneity of slopes (Green, Salkind, & Akey, 2000). When appropriate, LSD post hoc pairwise comparisons were used to examine the differences among the groups. For effect size, the partial eta squared (Green et al. 2000) statistic was calculated, and according to Green et al. (2000), 0.01, 0.06, and 0.14 represents small, medium, and large effect sizes, respectively. An alpha of $p<0.05$ was established a priori.

CHAPTER IV

Results

The pre- and post-intervention mean and standard deviations for all metabolic and performance measures ($\dot{V}O_2$peak, P_{peak}, T_{max}, RCP, PRCP, VT, and PVT) for all groups (CTL, HIIT, HMB-HIIT) are provided in Table 2. Table 3 provides the group mean and standard deviations for pre- to post-intervention body composition measures (BW, LSTM, total body fat mass, and BF).

Peak Oxygen Uptake ($\dot{V}O_2$peak)

The ANCOVA indicated a significant difference ($p=0.003$, $\eta^2=0.322$) among the group means for the posttest $\dot{V}O_2$peak values after adjusting for pre-test differences (Figure 2). The strength of the association (i.e., effect size, η^2) indicated that the treatment groups (CTL, HIIT, HMB-HIIT) accounted for 32% of the variance of the posttest $\dot{V}O_2$peak values, holding constant the pretest $\dot{V}O2$peak scores. The LSD pairwise comparisons indicated that the increase in $\dot{V}O_2$peak from pretest to post-testing was greater for the HMB-HIIT group than for the CTL ($p=0.001$) and the HIIT groups ($p=0.032$), however, no differences were found between HIIT and CTL groups ($p=0.09$). The group means ($\pm$SEM) for the posttest $\dot{V}O_2$peak values, adjusted for initial differences in pretest scores, are shown in Figure 2.

Table 2. Metabolic and Performance Measures for Pre- and Post-Supplementation

Measure	Control (*n*=8)		HIIT (*n*=13)		HMB-HIIT (*n*=13)	
	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest
VO_2peak ($mL\cdot kg^{-1}\cdot min^{-1}$)	39.1±4.5	38.9±4.0	38.9±3.4	40.3±2.6	39.8±6.7	42.7±5.1
P_{peak} (W)	218.75±41.7	215.63±32.6	221.15±46.6	236.54±48.5	226.92±56.3	246.15±54.8
T_{max} (min)	12.5±2.9	12.2±2.3	13.0±3.8	14.2±3.7	13.6±4.7	14.9±4.5
RCP ($mL\cdot kg^{-1}\cdot min^{-1}$)	30.49±5.0	28.71±2.7	29.33±3.1	31.88±2.2	32.16±4.2	33.73±3.8
PRCP (W)	175.25±38.8	167±24.8	168.35±36.0	184.96±33.5	182.62±33.6	196.5±35.1
VT ($mL\cdot kg^{-1}\cdot min^{-1}$)	30.15±5.01	29.23±4.5	28.63±3.1	29.04±4.1	27.84±4.8	31.66±3.7
PVT (W)	156.25±17.7	153.13±28.2	159.6±40.2	169.2±37.0	161.54±39.02	184.62±37.6

Values are means±SD. HIIT, high intensity interval training; HMB, β-hydroxy-β-methylbutyrate; VO_2peak, peak oxygen uptake; P_{peak}, peak power achieved; Tmax, time to exhaustion during graded exercise test; RCP, respiratory-compensation point; VT, ventilatory threshold;

Table 3. Body Composition Measures for Pre- and Post-Supplementation

Measure	Control (*n*=8)		HIIT (*n*=13)		HMB-HIIT (*n*=13)	
	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest
Body Weight (kg)	76.3±12.8	75.5±12.7	74.9±16.6	75.2±16.3	72.4±9.9	72.5±10.0
Lean Soft Tissue Mass (kg)	56.5±11.7	56.4±10.7	58.4±16.6	58.6±16.6	52.2±10.9	52.2±10.9
Total Body Fat Mass (kg)	15.9±7.0	14.3±8.4	13.3±4.8	13.2±4.6	16.9±5.3	17.0±5.4
Body Fat %	22.4±8.1	22.0±2.8	19.7±8.6	19.5±8.4	24.8±8.1	24.6±7.7

Values are means ±SD. HIIT, high intensity interval training; HMB, β-hydroxy-β-methylbutyrate.
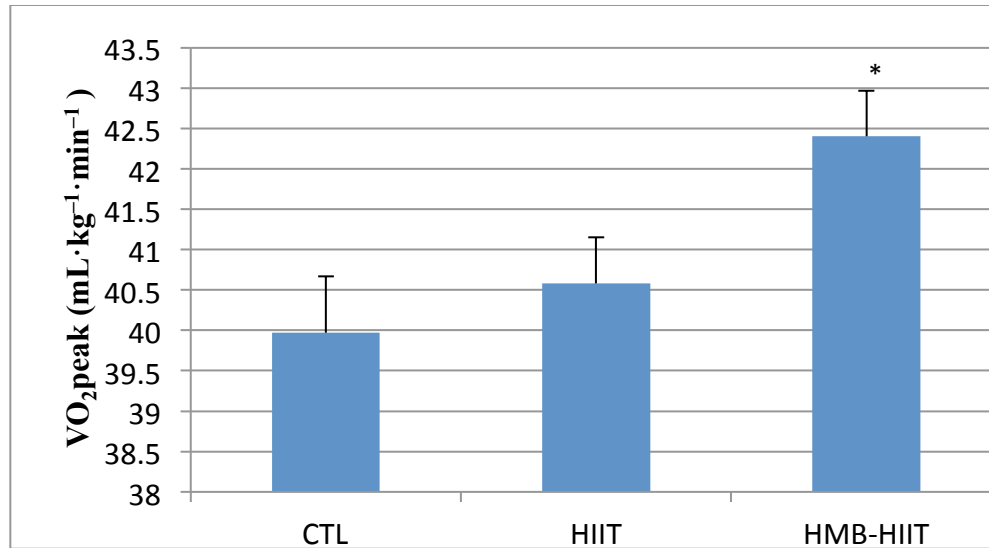
Figure 2. $\dot{V}O_2$peak obtained during graded exercise test. Mean values (+SEM) for posttest $\dot{V}O_2$peak scores adjusted for the initial differences in pretest VO_2peak (covariate; adjusted pretest mean=39.3). *HMB significantly greater than HIIT (p=0.032) and CTL (p=0.001)

Peak Power (P$_{peak}$)

The ANCOVA indicated a significant difference (p=0.013, η^2=0.251) among the group means for the posttest P$_{peak}$ values after adjusting for pre-test differences (Figure 3). The strength of the association (i.e., effect size, η^2) indicated that the treatment groups (CTL, HIIT, HMB-HIIT) accounted for 25% of the variance of the posttest P$_{peak}$ values, holding constant the pretest P$_{peak}$ scores. The LSD pairwise comparisons indicated that the increase in P$_{peak}$ from pretest to post-testing was greater for the HMB-HIIT (p=0.04) and HIIT (p=0.018) groups than for the CTL group, however, no differences were found between HMB-HIIT and HIIT groups (p=0.51). The group means (±SEM) for the posttest P$_{peak}$ values, adjusted for initial differences in pretest scores, are shown in Figure 3.

Figure 3. Peak power (P_{peak}) obtained during graded exercise test. Mean values (+SEM) for posttest P_{peak} scores adjusted for the initial differences in pretest P_{peak} (covariate; adjusted pretest mean=222.79). *Indicates significantly different than CTL (HIIT, p=0.018; HMB-HIIT, *p*=0.04)

Time to Exhaustion (T_{max})

The ANCOVA indicated a significant difference (*p*=0.002, η^2=0.35) among the group means for the posttest T_{max} values after adjusting for pre-test differences (Figure 4). The strength of the association (i.e., effect size, η^2) indicated that the treatment groups (CTL, HIIT, HMB-HIIT) accounted for 35% of the variance of the posttest T_{max} values, holding constant the pretest T_{max} scores. The LSD pairwise comparisons indicated that the increase in T_{max} from pretest to post-testing was greater for the HMB-HIIT (*p*=0.001) and HIIT (*p*=0.002) groups than for the CTL group, however, no differences were found between HMB-HIIT and HIIT groups (*p*=0.62). The group means (±SEM) for the posttest T_{max} values, adjusted for initial differences in pretest scores, are shown in Figure 4.

Figure 4. Time to exhaustion (T_{max}) for the graded exercise test. Mean values (+SEM) for posttest T_{max} scores adjusted for the initial differences in pretest T_{max} (covariate; adjusted pretest mean=13.11). *Indicates significantly different than CTL (HIIT, p=0.002; HMB-HIIT, *p*=0.001)

Respiratory Compensation Point (RCP)

The ANCOVA indicated a significant difference (*p*<0.001, η^2=0.436) among the group means for the posttest RCP values after adjusting for pre-test differences (Figure 5). The strength of the association (i.e., effect size, η^2) indicated that the treatment groups (CTL, HIIT, HMB-HIIT) accounted for 44% of the variance of the posttest RCP values, holding constant the pretest RCP scores. The LSD pairwise comparisons indicated that the increase in RCP from pretest to post-testing was greater for the HMB-HIIT (*p*<0.001) and HIIT (*p*<0.001) groups than for the CTL group, however, no differences were found between HMB-HIIT and HIIT groups (*p*=0.77). The group means (±SEM) for the posttest RCP values, adjusted for initial differences in pretest scores, are shown in Figure 5.
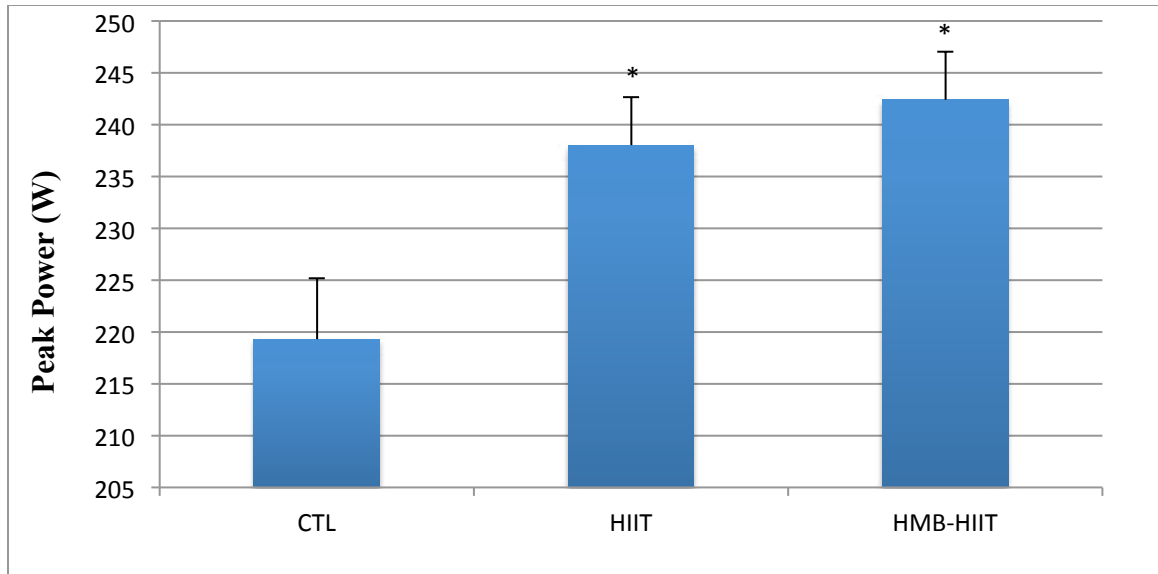
Figure 5. Respiratory compensation point (RCP). Mean values (+SEM) for posttest RCP scores adjusted for the initial differences in pretest RCP (covariate; adjusted pretest mean=30.69). *Indicates significantly different than CTL (HIIT, p<0.001; HMB-HIIT, p<0.001)

Power at Respiratory Compensation Point (PRCP)

The ANCOVA indicated a significant difference (p=0.001, η2=0.375) among the group means for the posttest PRCP values after adjusting for pre-test differences (Table 2, Figure 6). The strength of the association (i.e., effect size, η^2) indicated that the treatment groups (CTL, HIIT, HMB-HIIT) accounted for 38% of the variance of the posttest PRCP values, holding constant the pretest PRCP scores. The LSD pairwise comparisons indicated that the increase in PRCP from pretest to post-testing was greater for the HMB-HIIT (p<0.001) and HIIT (p<0.001) groups than for the CTL group, however, no differences were found between HMB-HIIT and HIIT groups (p=0.97). The group means (±SEM) for the posttest PRCP values, adjusted for initial differences in pretest scores, are shown in Figure 6.
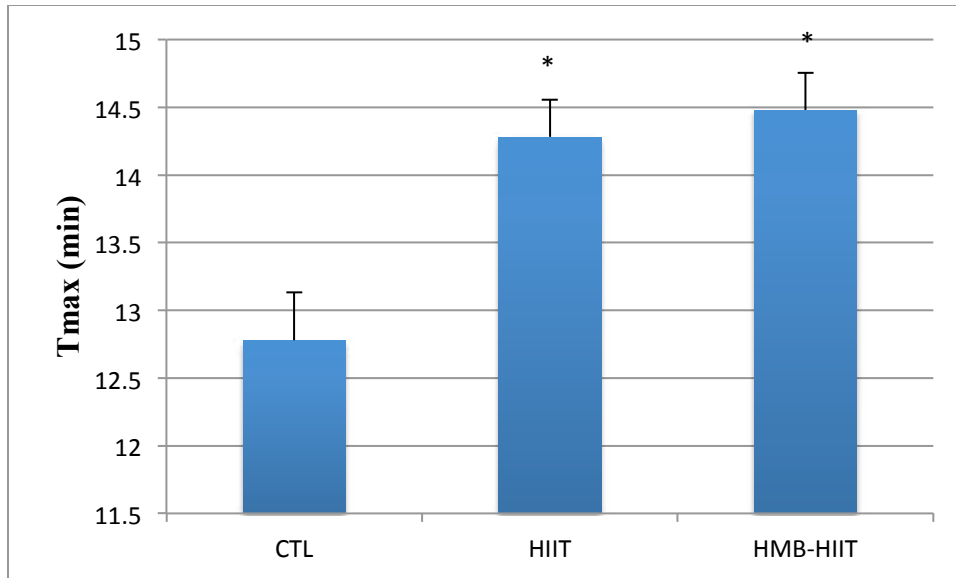
50

Figure 6. Power at respiratory compensation point (PRCP). Mean values (+SEM) for posttest PRCP scores adjusted for the initial differences in pretest PRCP (covariate; adjusted pretest mean=175.43). *Indicates significantly different than CTL (HIIT, p=0.001; HMB-HIIT, p=0.001)

Ventilatory Threshold (VT)

The ANCOVA indicated a significant difference ($p=0.016$, $\eta2=0.24$) among the group means for the posttest VT values after adjusting for pre-test differences (Figure 7). The strength of the association (i.e., effect size, η^2) indicated that the treatment groups (CTL, HIIT, HMB-HIIT) accounted for 24% of the variance of the posttest VT values, holding constant the pretest VT scores. The LSD pairwise comparisons indicated that the increase in VT from pretest to post-testing was greater for the HMB-HIIT group than for the CTL ($p=0.012$) and the HIIT groups ($p=0.017$), however, no differences were found between HIIT and CTL groups ($p=0.6$). The group means (±SEM) for the posttest VT values, adjusted for initial differences in pretest scores, are shown in Figure 7.
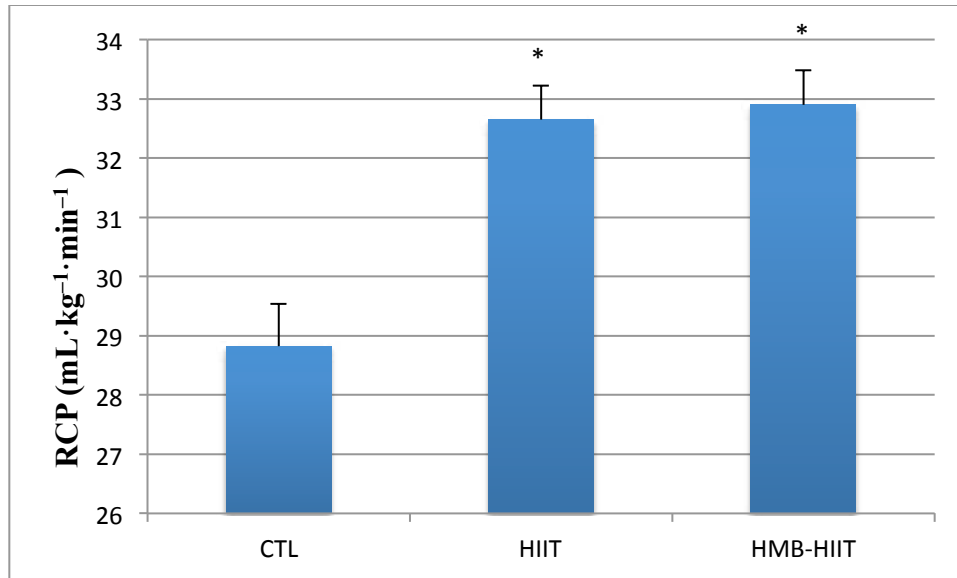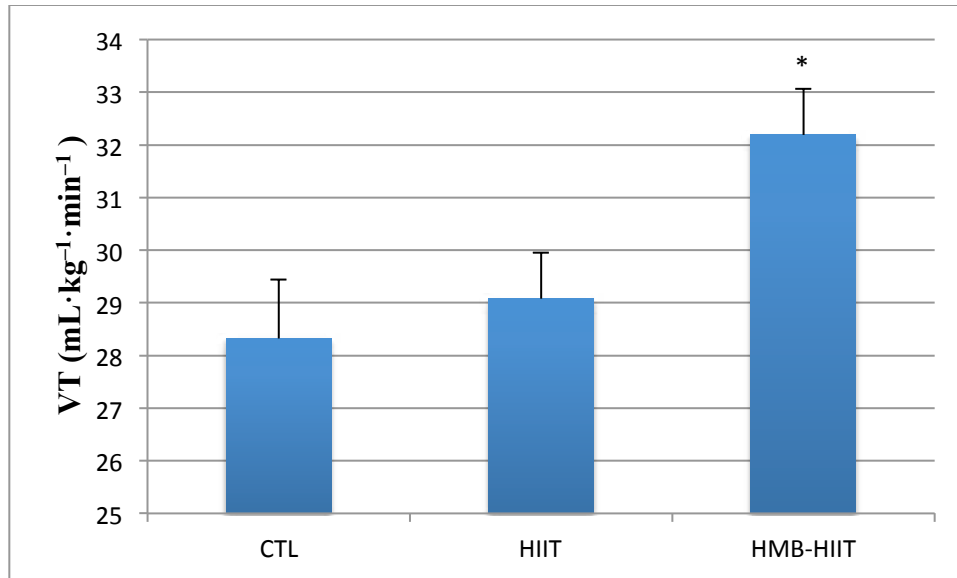
Figure 7. Ventilatory Threshold (VT). Mean values (+SEM) for posttest VT scores adjusted for the initial differences in pretest VT (covariate; adjusted pretest mean=28.68). *HMB-HIIT significantly greater than HIIT (p=0.017) and CTL (p=0.012)

Power at Ventilatory Threshold (PVT)

The ANCOVA indicated a significant difference (p=0.009, η^2=0.267) among the group means

for the posttest PVT values after adjusting for pre-test differences (Figure 8). The strength of the

association (i.e., effect size, η^2) indicated that the treatment groups (CTL, HIIT, HMB-HIIT)

accounted for 27% of the variance of the posttest PVT values, holding constant the pretest PVT

scores. The LSD pairwise comparisons indicated that the increase in PVT from pretest to post-

testing was greater for the HMB-HIIT group than for the CTL (p=0.004) and the HIIT groups

(p=0.027), however, no differences were found between HIIT and CTL groups (p=0.277). The

group means (±SEM) for the posttest PVT values, adjusted for initial differences in pretest
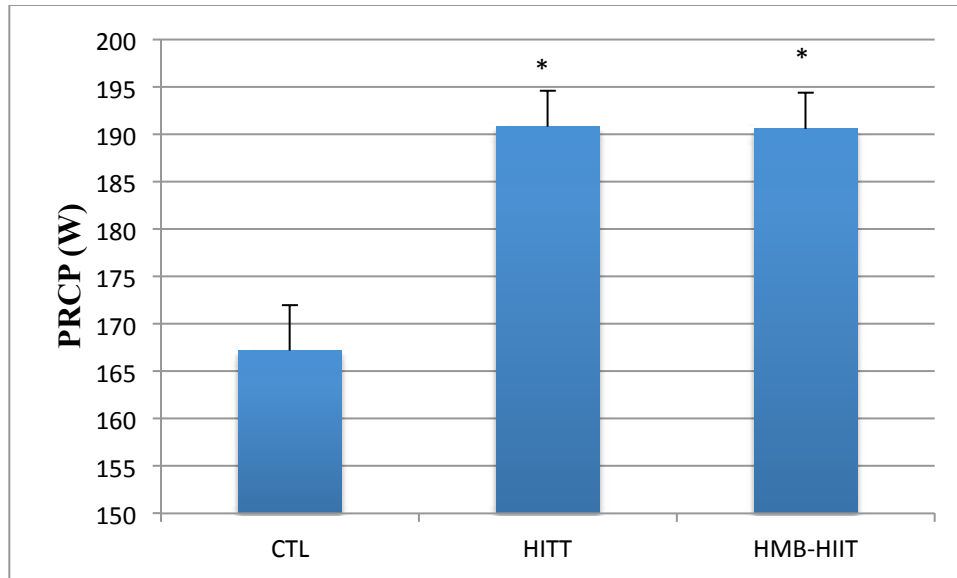
scores, are shown in Figure 8.
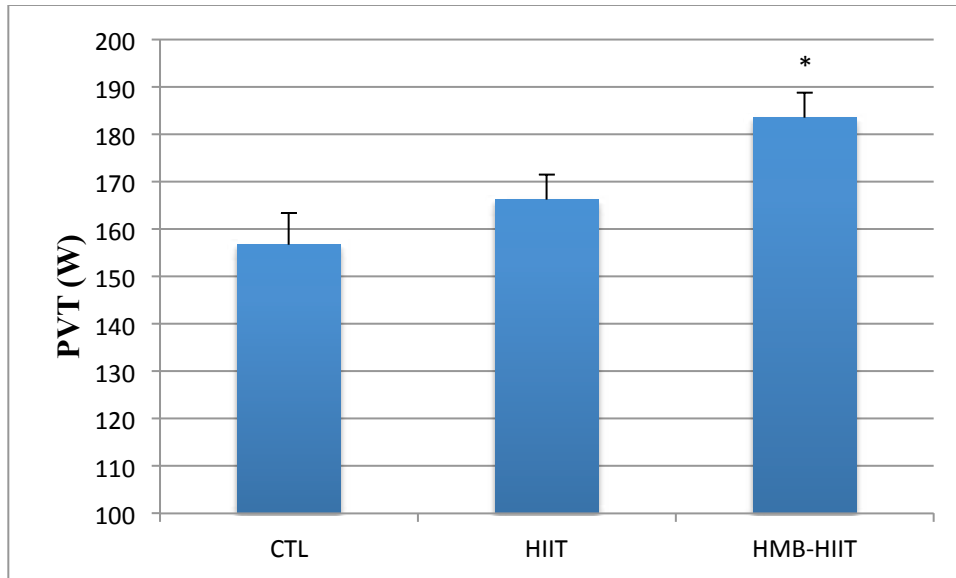
Figure 8. Power at ventilatory threshold (PVT). Mean values (+SEM) for posttest PVT scores adjusted for the initial differences in pretest PVT (covariate; adjusted pretest mean=160.29). *HMB significantly greater than HIIT (p=0.027) and CTL (p=0.004)

Body Composition

The ANCOVA indicated no significant difference for body mass (p=0.31, η^2=0.074) percent body fat (p=0.88, η^2=0.009), and lean soft tissue mass (p=0.247, η^2=0.089) between the all groups (Table 3).

Training Volume

An independent-samples t-test was performed to compare total training volume for the HIIT and HMB-HIIT groups. There was no significant difference (p=0.31) between training volumes for HIIT (1436.98±309.6 kJ) and HMB-HIIT (1456.75±378.55 kJ).

Dietary Analysis

An independent-samples t-test was performed to compare the dietary intake of total energy intake and the amount of the amino acid, leucine, consumed for the HIIT and HMB-HIIT groups. There was no significant difference for energy intake (p=0.159;HIIT,

2398.65±619.5Kcal; HMB-HIIT, 2011±620.2Kcal) or leucine intake (p=0.561;HIIT, 3.32±1.74g; HMB-HIIT, 3.85±2.13g) between the two treatment groups.

Supplementation Compliance

Placebo or HMB intake was recorded on individual intake logs, which were returned to the laboratory and monitored and resulted in 99% compliance. In addition, blood plasma HMB concentrations were analyzed by gas chromatography-mass spectrometry and performed by Metabolic Technologies Inc. using methods previously described to assess compliance and validate HMB in supplement packets (Nissen et al., 1990). HMB levels significantly (p=0.043) increased in the HMB-HIIT group (2.49±2.97 nmol·ml^{-1}) with no change (p=0.68) in the HIIT group (-0.08±0.21 nmol·ml^{-1}).

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-methylbutyrate (HMB) supplementation and cycling high-intensity interval training (HIIT) on metabolic and performance measures in young men and women. The primary findings support the use of HIIT as a training method to improve aerobic fitness. Furthermore, the results of the current study suggest that HMB supplementation significantly improved the benefits of the 4-week HIIT program on some of the aerobic and metabolic measures when compared to HIIT with the placebo.

The HIIT protocol used in the current study (Figure 1) resulted in a 4 to 11% increase in aerobic performance measures ($\dot{V}O_2$peak, P_{peak}, T_{max}; Table 2). In support, Smith et al. (2009) reported a 9% to 11% increase in $\dot{V}O_2$peak and T_{max} after 3-weeks of HIIT using a similar protocol. In agreement, several studies have reported 7 to10% increases in $\dot{V}O_2$peak using HIIT protocols in college-aged participants (Duffield, Edge, & Bishop, 2006; Helgerud et al., 2007; Talanian, Galloway, Heigenhauser, Bonen, & Spriet, 2007). Although previous studies utilizing this method of HIIT involved training 5 days per week, Jourkesh et al. (2011) also reported a significant increase in T_{max} after 3 weeks of fractal periodized HIIT and a significant increase in $\dot{V}O_2$peak after 6 weeks with training 3 times per week.

Furthermore, in the current investigation, supplementation with HMB, in addition to HIIT, significantly increased $\dot{V}O_2$peak (Table 2, Figure 2) greater than training alone. The present results are in agreement with Lamboley et al. (2007) who reported a 15% increase in $\dot{V}O_2$max after 5 weeks of a running HIIT program while supplementing 3grams per day of Calcium β-hydroxy-β-methylbutyrate (CaHMB) in college age men and women. In contrast,

previous studies, which involved supplementation of CaHMB while endurance training, found

no increase in $\dot{V}O_2$peak with 2 to 6 weeks of supplementation (Knitter et al., 2000; Vukovich &

Dreifort, 2001). In a cross-over design, Vukovich and Dreifort (2001), examined the effect of

CaHMB supplementation in endurance-trained cyclists, and reported no significant increase in

$\dot{V}O_2$peak in these highly trained athletes, however, there was a significant increase (3.6%) in the

time reach to $\dot{V}O_2$peak (T_{max}). The increase in T_{max} observed in Vukovich and Dreifort (2001),

was smaller than our observed 8% increase in younger untrained men and women (Table 2). The

discrepancy between our study and previous endurance studies (Knitter et al., 2000; Vukovich &

Dreifort, 2001) examining HMB, could be due to the participants used in the investigation. It

has been suggested that active men and women who are unaccustomed to HIIT may benefit more

from HMB supplementation than trained athletes who are accustomed to HIIT (Lamboley et al.,

2007). The participants in the current study were unfamiliar with HIIT, which may explain why

our results were similar to Lamboley et al. and not Vukovich et al. who used trained endurance

athletes. However, Knitter et al. also examined individuals unaccustomed to HIIT, but reported

no additive effect of HMB supplement on endurance performance measures. Therefore, training

status and previous experience with HIIT could have influenced the current results while

explaining differences from previous investigations.

The type of training and manner in which it was conducted may have played an

additional role in the observed physiological adaptations. The differences reported by Lamboley

et al. and our findings versus other studies may be due to the fact that individualized HIIT

programs were developed based on each participant's baseline fitness level and monitored

throughout the 28 days of training, while it was unclear what endurance program was used in

other studies(Knitter et al., 2000; Vukovich & Dreifort, 2001) as they did not describe in detail

the exercise interventions. Therefore, the difference in results by Knitter (2000) and Vukovich et al. (2001) in comparison to Lamboley et al. (2007) and our data may be that the training programs were insufficient at stimulating physiological adaptation (J. M. Wilson et al., 2013; J. M. Wilson et al., 2013; Zanchi et al., 2011).

Fatigue threshold measures, such as ventilatory threshold (VT), respiratory compensation point (RCP), and onset of blood lactate accumulation (OBLA), have been used as non-invasive measures of health, performance, and in the evaluation of the efficacy of endurance training and/or nutritional supplementation (Lamboley et al., 2007; J. Stout et al., 2007; Zoeller, Stout, O'kroy, Torok, & Mielke, 2007). Further, the measurement of specific fatigue thresholds during a graded exercise test, like VT and RCP, may be useful for demarcating the heavy or severe exercise intensity domains, respectively (Bergstrom et al. 2013). For example, VT has been associated with exercise intensity that results in excessive CO_2 production from the bicarbonate buffering of hydrogen ions (Gaesser & Poole, 1996; Wasserman, Beaver, & Whipp, 1990), while RCP has been associated with severe intensity exercise which leads to excessive minute ventilation resulting from hyperkalemia (Bergstrom et al., 2013; Paterson et al., 1990). The measurement of fatigue thresholds (VT, RCP), therefore, may provide possible mechanistic explanation for aerobic performance changes from training or nutritional interventions. Additionally, assessment of the exercise intensity domains, heavy (VT), severe (RCP) and maximal ($\dot{V}O_2$peak), during a graded exercise test may improve the sensitivity of detecting the potential effects on aerobic performance from various exercise and or nutritional interventions due to different mechanisms.

In the current study, the four-week HIIT program resulted in a 6.3% increase in power output at ventilatory threshold (PVT) (Table 2) which is similar to Smith et al. (2009) who

reported a 10.2 % increase using a comparable three-week HIIT cycling protocol in untrained college aged men. In addition, our study demonstrated an 8.6% increase in RCP which was very similar to the changes reported by Lamboley et al (2007) of an 8.5% increase from 5 weeks of HIIT on a treadmill. Our data, along with Smith et al. (2009) and Lamboley et al (2007), supports previous studies that demonstrate HIIT consistently improves aerobic performance measures (Berger, Campbell, Wilkerson, & Jones, 2006; Eddy, Sparks, & Adelizi, 1977; Helgerud et al., 2007).

The addition of HMB to the four weeks of HIIT (HMB-HIIT) resulted in a ~14% increase in VT which was significantly greater than HIIT alone (Table 2, Figure 7). However, there was no difference between HMB-HIIT and HIIT groups for changes in RCP (Table 2, Figure 5). While our findings are similar to percent change (+11.1%) in VT as a previous study reported, Lamboley et al. (2007) discovered no significant difference between HMB-HIIT and HIIT groups. Conversely, Lamboley et al. (2007) reported significantly greater changes in RCP for HMB-HIIT compared to HIIT, whereas the current investigation resulted in similar changes between groups.   Furthermore, Vukovich and Dreifort (2001) reported a 9.1% increase in OBLA after two weeks of CaHMB supplementation in elite cyclists.   Previous researchers have used OBLA as a method to identify the crossover point between moderate and heavy exercise intensities denoted by blood lactate concentrations greater than 4 millimoles per liter during an incremental exercise test (Heck et al., 1985). With previous evidence supporting OBLA and VT as fatigue thresholds representing similar exercise domains, the increases in exercise intensity at OBLA (+9.1%) reported by Vukovich and Dreifort (2001) and the increase in VT (+14%) observed in our study (Table 2) may reflect similar physiological adaptations. Our results, along with Vukovich and Dreifort (2001) and Lamboley et al. (2007), suggest that HMB may augment

the beneficial effects of HIIT on aerobic performance by increasing fatigue threshold measures that reflect the physiological response to moderate and/or severe intensity exercise.

The observed physiological changes in aerobic performance from HIIT have been shown to improve $\dot{V}O_2$peak, muscle buffering capacity, and whole body fat oxidation (Edge, Bishop, & Goodman, 2006; Laursen & Jenkins, 2002; Weston, Zhou, Weatherby, & Robertson, 1997). Further, the improved aerobic power associated with HIIT has been linked to an up-regulation of glycolytic enzymes, as well as, increased mitochondrial density and blood flow (Henriksson, 1992; Krustrup, Söderlund, Mohr, & Bangsbo, 2004). Recently, it has been suggested that HMB supplementation may improve fatty acid oxidation, adenosine monophosphate kinase (AMPK), Sirt1, and Sirt3 activity in muscle cells (Bruckbauer et al., 2012; Pinheiro et al., 2012). Sirt1, Sirt3, and AMPK have been shown to augment mitochondrial biogenesis, lipolysis, energy metabolism and the reactive oxygen defense system (Hardie & Sakamoto, 2006; Verdin, Hirschey, Finley, & Haigis, 2010). In support, Stancliffe (2012) reported that HMB mediated mitochondrial biogenesis in murine myotubes. Additionally, Pinheiro et al. (2012) reported that 28 days of CaHMB administration in male Wistar rats resulted in significantly increased intramuscular ATP and glycogen content. While speculative, HMB supplementation may have enhanced the effects of HIIT by improving mitochondrial biogenesis, fat oxidation, and energy metabolism and capacity. However, more research is needed to support these proposed mechanisms in humans.

In conclusion, our findings reinforce the use of HIIT as an effective training stimulus for improving aerobic performance. In addition, the use of HMB supplementation, in combination with HIIT, appeared to result in greater changes in $\dot{V}O_2$peak and VT than HIIT alone. While more research is needed, the current investigation suggests that in college age men and women,

the use of HMB supplementation may enhance the benefits of HIIT on aerobic performance

measures.

APPENDIX A

UCF IRB LETTER OF APPROVAL

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

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APPENDIX B

NEW ENGLAND IRB APPROVAL LETTER

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

- 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, MCJ, CIM, CIP
Lead Administrator

Copy: NEIRB Chair
        Enclosures

APPENDIX C

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

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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

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

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.

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

APPENDIX D

MEDICAL HISTORY QUESTIONNAIRE

Confidential Medical and Activity History Questionnaire

Participant #_____

When was your last physical examination? _____

1. **List any medications, herbals or supplements you currently take or have taken the last month**:

   Medication   Reason for medication
   _____   _____
   _____   _____
   _____   _____
   _____   _____

2. **Are you allergic to any medications? If yes, please list medications and reaction**.

3. **Please list any allergies, including food allergies that you may have?**

4. **Have you ever been hospitalized? If yes, please explain**.

   Year of hospitalization   Reason
   _____   _____
   _____   _____
   _____   _____

5. **Illnesses and other Health Issues**

   List any chronic (long-term) illnesses that have caused you to seek medical care.

1

Have you ever had (or do you have now) any of the following. Please circle
questions that you do not know the answer to.

Sickle cell anemia	yes	no
Cystic fibrosis	yes	no
Water retention problems	yes	no
Heart pacemaker	yes	no
Epilepsy	yes	no
Convulsions	yes	no
Dizziness/fainting/unconsciousness	yes	no
Asthma	yes	no
Shortness of breath	yes	no
Chronic respiratory disorder	yes	no
Chronic headaches	yes	no
Chronic cough	yes	no
Chronic sinus problem	yes	no
High blood pressure	yes	no
Heart murmur	yes	no
Heart attack	yes	no
High cholesterol	yes	no
Diabetes mellitus or insipidus	yes	no
Rheumatic fever	yes	no
Emphysema	yes	no
Bronchitis	yes	no
Hepatitis	yes	no
Kidney disease	yes	no
Bladder problems	yes	no
Tuberculosis (positive skin test)	yes	no
Yellow jaundice	yes	no
Auto immune deficiency	yes	no
Anemia	yes	no
Endotoxemia	yes	no
Thyroid problems	yes	no
Hyperprolactinemia	yes	no
Anorexia nervosa	yes	no
Bulimia	yes	no
Stomach/intestinal problems	yes	no
Arthritis	yes	no
Back pain	yes	no
Gout	yes	no
Hepatic encephalopathy	yes	no
Mania	yes	no
Hypermania	yes	no
Monosodium glutamate hypersensitivity	yes	no
Seizure disorders	yes	no

2

Any others (specify):_____

_____

_____

Do you smoke cigarettes or use any other tobacco products?	yes	no
Do you have a history of drug or alcohol dependency?	yes	no
Do you ever have any pain in your chest?	yes	no
Are you ever bothered by racing of your heart?	yes	no
Do you ever notice abnormal or skipped heartbeats?	yes	no
Do you ever have any arm or jaw discomfort, nausea, Or vomiting associated with cardiac symptoms?	yes	no
Do you ever have difficulty breathing?	yes	no
Do you ever experience shortness of breath?	yes	no
Do you ever become dizzy during exercise?	yes	no
Are you pregnant?	yes	no
Is there a chance that you may be pregnant?	yes	no
Have you ever had any tingling or numbness in your arms or legs?	yes	no
Has a member of your family or close relative died of heart problems or sudden death before the age of 50?	yes	no
Has a health care practitioner ever denied or restricted your participation in sports for any problem	yes	no

If yes, please explain: _____

_____

Are you presently taking any nutritional supplements or ergogenic aids? (if yes, please detail. _____

_____

_____

_____

_____

3

APPENDIX E

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of <u>any other reason</u> why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to <u>all</u> PAR-Q questions, you can be reasonably sure that you can:
- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:
- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

<u>Informed Use of the PAR-Q</u>: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____   DATE_____

SIGNATURE OF PARENT _____   WITNESS _____
or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

APPENDIX F

NEW ENGLAND IRB APPROVED RECRUITMENT FLYER

Volunteers Needed for Research Study

Want to learn a new way to endurance train?

We need participants for a research study:
"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"

Description of Project: We are investigating the effect of how a new version of the sport supplement HMB and high-intensity interval training might change the musculature of the leg and improve cardiovascular and neuromuscular performance.

Who is Eligible? Men and Women between the Ages of 18-35 who regularly Run or Bike

What will you be asked to do? Complete 2 Pre- and 2 Post-Testing sessions. 4 weeks of High Intensity Interval Training on a stationary bike (approx. 30 min per session)

Compensation: Participants will receive $100 for completion of the study.

To learn more, contact the principal investigator of the study, Ned Robinson, at 407-823-2367 or humanperformancelab@ucf.edu.
This research is conducted under the direction of Dr. Jeffrey Stout, Educational and Human Sciences Department.

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REFERENCES

Beaver, W. L., Wasserman, K., & Whipp, B. J. (1986). A new method for detecting anaerobic threshold by gas exchange. *Journal of Applied Physiology, 60*(6), 2020-2027.

Berger, N. J., Campbell, I. T., Wilkerson, D. P., & Jones, A. M. (2006). Influence of acute plasma volume expansion on VO2 kinetics, VO2 peak, and performance during high-intensity cycle exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985), 101*(3), 707-714. doi:10.1152/japplphysiol.00154.2006

Bergstrom, H. C., Housh, T. J., Zuniga, J. M., Traylor, D. A., Camic, C. L., Lewis Jr, R. W., et al. (2013). The relationships among critical power determined from a 3-min all-out test, respiratory compensation point, gas exchange threshold, and ventilatory threshold. *Research Quarterly for Exercise and Sport, 84*(2), 232-238.

Bruckbauer, A., Zemel, M. B., Thorpe, T., Akula, M. R., Stuckey, A. C., Osborne, D., et al. (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; 10.1186/1743-7075-9-77

Caiozzo, V. J., Davis, J. A., Ellis, J. F., Azus, J. L., Vandagriff, R., Prietto, C., et al. (1982). A comparison of gas exchange indices used to detect the anaerobic threshold. *Journal of Applied Physiology, 53*(5), 1184-1189.

Carbone, J. W., McClung, J. P., & Pasiakos, S. M. (2012). Skeletal muscle responses to negative energy balance: Effects of dietary protein. *Advances in Nutrition (Bethesda, Md.), 3*(2), 119-126. doi:10.3945/an.111.001792; 10.3945/an.111.001792

Churchward-Venne, T. A., Breen, L., Di Donato, D. M., Hector, A. J., Mitchell, C. J., Moore, D. R., et al. (2014). Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: A double-blind, randomized trial. *The American Journal of Clinical Nutrition, 99*(2), 276-286. doi:10.3945/ajcn.113.068775; 10.3945/ajcn.113.068775

Daussin, F. N., Zoll, J., Dufour, S. P., Ponsot, E., Lonsdorfer-Wolf, E., Doutreleau, S., et al. (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-R272.

Duffield, R., Edge, J., & Bishop, D. (2006). Effects of high-intensity interval training on the response during severe exercise. *Journal of Science and Medicine in Sport, 9*(3), 249-255.

Eddy, D. O., Sparks, K. L., & Adelizi, D. A. (1977). The effects of continuous and interval training in women and men. *European Journal of Applied Physiology and Occupational Physiology, 37*(2), 83-92.

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.

Flakoll, P., Sharp, R., Baier, S., Levenhagen, D., Carr, C., & Nissen, S. (2004). Effect of β-hydroxy-β-methylbutyrate, arginine, and lysine supplementation on strength, functionality, body composition, and protein metabolism in elderly women. *Nutrition, 20*(5), 445. doi:10.1016/j.nut.2004.01.009

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.

Gaesser, G. A., & Poole, D. C. (1996). The slow component of oxygen uptake kinetics in humans. *Exercise and Sport Sciences Reviews, 24*(1), 35-70.

Gravettier, & Wallnau. (1996). *Statistics for the behavioral sciences* (4th ed.). St. Paul, MN: West Publishing Co.

Green, S., Salkind, N., & Akey, T. (2000). Methods for controlling type I error across multiple hypothesis tests. *Using SPSS for Windows: Analysing and Understanding Data, 2nd Edn.New Jersey: Prentice-Hall, ,* 395-396.

Hardie, D. G., & Sakamoto, K. (2006). AMPK: A key sensor of fuel and energy status in skeletal muscle. *Physiology, 21*(1), 48-60.

Heck, H., Mader, A., Hess, G., Mucke, S., Muller, R., & Hollmann, W. (1985). Justification of the 4-mmol/l lactate threshold. *International Journal of Sports Medicine, 6*(3), 117-130. doi:10.1055/s-2008-1025824

Helgerud, J., Hoydal, K., Wang, E., Karlsen, T., Berg, P., Bjerkaas, M., et al. (2007). Aerobic high-intensity intervals improve VO~ 2~ m~ a~ x more than moderate training. *Medicine and Science in Sports and Exercise, 39*(4), 665.

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

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

Huck, S. W., & McLean, R. A. (1975). Using a repeated measures ANOVA to analyze the data from a pretest-posttest design: A potentially confusing task. *Psychological Bulletin, 82*(4), 511.

Jacobs, R. A., Boushel, R., Wright-Paradis, C., Calbet, J. A., Robach, P., Gnaiger, E., et al. (2013). Mitochondrial function in human skeletal muscle following high-altitude exposure. *Experimental Physiology, 98*(1), 245-255.

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

Jourkesh, M., Ahmaidi, S., Keikha, B., 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), 403-404-413.

Katsanos, C. S., Kobayashi, H., Sheffield-Moore, M., Aarsland, A., & Wolfe, R. R. (2006). A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *American Journal of*

Physiology.Endocrinology and Metabolism, 291(2), E381-7. doi:10.1152/ajpendo.00488.2005

Keating, S. E., Machan, E. A., O'Connor, H. T., Gerofi, J. A., Sainsbury, A., Caterson, I. D., et al. (2014). Continuous exercise but not high intensity interval training improves fat distribution in overweight adults. *Journal of Obesity, 2014*

Kimura, K., Cheng, X. W., Inoue, A., Hu, L., Koike, T., & Kuzuya, M. (2014). B-hydroxy-β-methylbutyrate facilitates PI3K/akt-dependent mTOR and FoxO1/3a phosphorylations and alleviates TNF-α/IFN-γ-induced MuRF-1 expression in C2C12 cells. *Nutrition Research,*

Knitter, A., Panton, L., Rathmacher, J., Petersen, A., & Sharp, R. (2000). Effects of β-hydroxy-β-methylbutyrate on muscle damage after a prolonged run. *Journal of Applied Physiology, 89*(4), 1340-1344.

Koller, A., Mair, J., Schobersberger, W., Wohlfarter, T., Haid, C., Mayr, M., et al. (1998). Effects of prolonged strenuous endurance exercise on plasma myosin heavy chain fragments and other muscular proteins. cycling vs running. *The Journal of Sports Medicine and Physical Fitness, 38*(1), 10-17.

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, 447*(6), 855-866.

Lamboley, C. R., Royer, D., & Dionne, I. J. (2007). Effects of beta-hydroxy-beta-methylbutyrate on aerobic-performance components and body composition in college students. *International Journal of Sport Nutrition and Exercise Metabolism, 17*(1), 56-69.

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

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. *The Journal of Strength & Conditioning Research, 19*(3), 527-533.

Meyer, T., Lucia, A., Earnest, C. P., & Kindermann, W. (2005). A conceptual framework for performance diagnosis and training prescription from submaximal gas exchange parameters-theory and application. *International Journal of Sports Medicine, 26*(S 1), S38-S48.

Nissen, S., Van Koevering, M., & Webb, D. (1990). Analysis of β-hydroxy-β-methyl butyrate in plasma by gas chromatography and mass spectrometry. *Analytical Biochemistry, 188*(1), 17-19.

Nissen, S., Sharp, R., Ray, M., Rathmacher, J. A., Rice, D., Fuller, J. C., J., et al. (1996). Effect of leucine metabolite beta-hydroxy-beta-methylbutyrate on muscle metabolism during resistance-exercise training. *Journal of Applied Physiology (Bethesda, Md.: 1985), 81*(5), 2095-2104.

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.

Norton, L. E., & Layman, D. K. (2006). Leucine regulates translation initiation of protein synthesis in skeletal muscle after exercise. *The Journal of Nutrition, 136*(2), 533S-537S.

Oshima, Y., Tanaka, S., Miyamoto, T., Wadazumi, T., Kurihara, N., & Fujimoto, S. (1998). Effects of endurance training above the anaerobic threshold on isocapnic buffering phase during incremental exercise in middle-distance runners. 体力科學, *47*(1), 43-51.

Park, B., Henning, P. C., Grant, S. C., Lee, W. J., Lee, S., Arjmandi, B. H., et al. HMB attenuates muscle loss during sustained energy deficit induced by calorie restriction and endurance exercise. *Metabolism,* (0)

Paterson, D. J., Friedland, J. S., Bascom, D. A., Clement, I. D., Cunningham, D. A., Painter, R., et al. (1990). Changes in arterial K+ and ventilation during exercise in normal subjects and subjects with McArdle's syndrome. *The Journal of Physiology, 429*, 339-348.

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.

Pinheiro, C., Gerlinger-Romero, F., Guimarães-Ferreira, L., Souza-Jr, A., Vitzel, K., Nachbar, R., et al. (2012). Metabolic and functional effects of beta-hydroxy-beta-methylbutyrate (HMB) supplementation in skeletal muscle. *European Journal of Applied Physiology, 112*(7), 2531-2537. doi:10.1007/s00421-011-2224-5

Smith, A. E., Walter, A. A., Graef, J. L., Kendall, K. L., Moon, J. R., Lockwood, C. M., et al. (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.

Stancliffe, R. A. (2012). Role of beta-hydroxy-beta-methylbutyrate (HMB) in leucine stimulation of mitochondrial biogenesis and fatty acid oxidation.

Stout, J. R., Smith-Ryan, A. E., Fukuda, D. H., Kendall, K. L., Moon, J. R., Hoffman, J. R., et al. (2013). Effect of calcium β-hydroxy-β-methylbutyrate (CaHMB) with and without resistance training in men and women 65 yrs: A randomized, double-blind pilot trial. *Experimental Gerontology, 48*(11), 1303-1310.

Stout, J., Cramer, J., Zoeller, R., Torok, D., Costa, P., Hoffman, J., et al. (2007). Effects of β-alanine supplementation on the onset of neuromuscular fatigue and ventilatory threshold in women. *Amino Acids, 32*(3), 381-386.

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, 102*(4), 1439-1447.

Tremblay, A., Simoneau, J., & Bouchard, C. (1994). Impact of exercise intensity on body fatness and skeletal muscle metabolism. *Metabolism, 43*(7), 814-818.

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.

Vukovich, M. D., & Dreifort, G. D. (2001). Effect of [beta]-hydroxy [beta]-methylbutyrate on the onset of blood lactate accumulation and Vo2peak in endurance-trained cyclists. *The Journal of Strength & Conditioning Research, 15*(4), 491-497.

Wasserman, K., Beaver, W. L., & Whipp, B. J. (1990). Gas exchange theory and the lactic acidosis (anaerobic) threshold. *Circulation, 81*(1 Suppl), II14-30.

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.

Wilkinson, D. J., Hossain, T., Hill, D. S., Phillips, B. E., Crossland, H., Williams, J., et al. (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.

Wilson, G. J., Wilson, J. M., & Manninen, A. H. (2008). Effects of beta-hydroxy-beta-methylbutyrate (HMB) on exercise performance and body composition across varying levels of age, sex, and training experience: A review. *Nutr Metab (Lond), 5*, 1.

Wilson, J. M., Fitschen, P. J., Campbell, B., Wilson, G. J., Zanchi, N., Taylor, L., et al. (2013). International society of sports nutrition position stand: Beta-hydroxy-beta-methylbutyrate (HMB). *Journal of the International Society of Sports Nutrition, 10*(1), 1-14.

Wilson, J. M., Lowery, R. P., Joy, J. M., Walters, J. A., Baier, S. M., Fuller, J. C., et al. (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, 1*(1), 1-7.

Zanchi, N., Gerlinger-Romero, F., Guimarães-Ferreira, L., de Siqueira Filho, M., Felitti, V., Lira, F., et al. (2011). HMB supplementation: Clinical and athletic performance-related effects and mechanisms of action. *Amino Acids, 40*(4), 1015-1025. doi:10.1007/s00726-010-0678-0

Zoeller, R., Stout, J., O'kroy, J., Torok, D., & Mielke, M. (2007). Effects of 28 days of beta-alanine and creatine monohydrate supplementation on aerobic power, ventilatory and lactate thresholds, and time to exhaustion. *Amino Acids, 33*(3), 505-510.