Pre-exercise β-hydroxy-β-methylbutyrate free-acid supplementation improves work capacity recovery: a randomized, double-blinded, placebo-controlled study

Journal: *Applied Physiology, Nutrition, and Metabolism*

Manuscript ID: apnm-2017-0867.R1

Manuscript Type: Article

Date Submitted by the Author: 18-Jan-2018

Complete List of Authors:
- Correia, Ana Luiza; University of Brasília de Lima, Filipe; University of Brasília - UnB, College of Physical Education
- Bottaro, Martim; University of Brasília, College of Physical Education
- Vieira, Amilton; University of Brasília
- da Fonseca, Andrew; University of Brasília
- Lima, Ricardo; University of Brasília

Keyword:
- Muscle recovery, Exercise-induced muscle damage, HMB-FA, sports nutrition < nutrition, Supplementation

Is the invited manuscript for consideration in a Special Issue?: N/A
Pre-exercise β-hydroxy-β-methylbutyrate free-acid supplementation improves work capacity recovery: a randomized, double-blinded, placebo-controlled study

Ana Luiza Matias Correia¹, Filipe Dinato de Lima², Martim Bottaro³, Amilton Vieira⁴, Andrew Correa da Fonseca⁵, Ricardo Moreno Lima⁶

Corresponding author: Filipe Dinato de Lima
Address: Universidade de Brasília (UnB), Campus Universitário Darcy Ribeiro, Faculdade de Educação Física, Brasília – DF, Brazil. Zipcode: 70910-900. Phone: +55 61 984355610. Fax: +55 61 31072500. fdinatolima@gmail.com

¹ College of Physical Education, University of Brasilia, Brasília-DF, Brazil. analuiza.matias2@gmail.com
² College of Health Sciences, University of Brasilia, Brasília-DF, Brazil. fdinatolima@gmail.com
³ College of Physical Education, University of Brasilia, Brasília-DF, Brazil. martim.bottaro@gmail.com
⁴ College of Physical Education, University of Brasilia, Brasília-DF, Brazil. acmribeirao@gmail.com
⁵ College of Physical Education, University of Brasilia, Brasília-DF, Brazil. andrewcfonseca@gmail.com
⁶ College of Physical Education, University of Brasilia, Brasília-DF, Brazil. professorricardomoreno@gmail.com
Abstract

The purpose of this study was to investigate the effects of a single-dose of β-hydroxy-β-methylbutyrate free acid (HMB-FA) supplementation on muscle recovery after a high-intensity exercise bout. Twenty-three trained young males were randomly assigned to receive either a single-dose supplementation of 3g of HMB-FA (n = 12; age 22.8 ± 3.0 years) or placebo (PLA; n = 11; age 22.9 ± 3.1 years). A muscle damage protocol was applied 60 minutes after supplementation, and consisted of seven sets of 20 drop jumps from a 60-cm box with 2-min rest intervals between sets. Muscle swelling, countermovement jump (CMJ), maximal voluntary isometric torque (MVIT) and work capacity (WC) were measured before, immediately after, 24, 48 and 72 hours after the exercise protocol. Muscle swelling, CMJ and MVIT changed similarly in both groups after the exercise protocol (p < 0.001), but returned to pre-exercise levels after 24 hours in both groups. WC decreased similarly in both groups after the exercise protocol (p < 0.01). For HMB-FA, WC returned to pre-exercise level 24 hours after exercise protocol. However, on PLA, WC did not return to pre-exercise level even 72 hours after the exercise protocol. In summary, a single-dose of HMB-FA supplementation improved WC recovery after a high-intensity exercise bout. However, HMB-FA did not affect the time-course of muscle swelling, MVIT and CMJ recovery.

Keywords: Muscle recovery; Exercise-induced muscle damage; HMB-FA; Supplementation; Sports nutrition
Introduction

The goals of sports medicine professionals include enhancing peak athletic performance while reducing injuries risks during training and competitions. In this direction, sports nutrition plays an important role before, during, and after exercise bouts in order to improve muscle recovery, glycogen repletion, and successive muscle performance (Zoorob et al. 2013; Spriet 2014; Beck et al. 2015). According to the American College of Sports Medicine, the Dietitians of Canada, and the Academy of Nutrition and Dietetics, nutritional interventions during training and competitions should be planned to reduce fatigue and avoid deteriorations in performance outputs such as muscle strength and power, agility, and endurance (Thomas et al. 2016).

Several nutritional strategies have been examined as ergogenic aids to improve performance recovery, depending on the physiological requirements of each specific sport (Beelen et al. 2010). In this regard, the potential role of carbohydrate intake (Burke et al. 2011; Stellingwerff et al. 2011), supplementation with antioxidants (Heaton et al. 2017), sodium bicarbonate and beta-alanine (Painelli et al. 2013), as well as protein intake (Jager et al. 2017) in improving performance recovery have been widely investigated. A metabolite of leucine named β-hydroxy-β-methylbutyrate (HMB) has also been reported to have positive anabolic effects on protein metabolism, improving protein synthesis and inhibiting protein breakdown by simulating mTOR pathway and increased growth hormone release (Wilson et al. 2013a; Holecek 2017). In terms of muscle recovery, it has been demonstrated that HMB acts as a precursor for cholesterol synthesis, which is used to repair damaged muscle cell membranes (Holecek 2017). HMB was primarily studied and commercialized as the calcium salt, with positive (van Someren et al. 2005; Wilson et al. 2009) or no effects (Hoffman et al. 2004; Nunan et al. 2010) on muscle performance observed in previous reports. Recently, a
new free acid HMB formula (HMB-FA) has been introduced, which has been suggested to promote greater effects compared to the traditional calcium salt formula (Fuller et al. 2011).

In fact, HMB-FA has been shown to produce higher plasma concentrations (+185%) in a shorter period of time (120 min for HMB-Ca vs. 30min for HMB-FA), and yields greater intra-muscular bioavailability (+25%) than the traditional calcium salt form (Fuller et al. 2011; Fuller et al. 2015). However, little data exist examining the effects of HMB-FA supplementation on muscle performance during recovery from an acute bout of exercise.

A recent systematic review (Silva et al. 2017) showed that only two studies have examined the effect of HMB-FA on muscle recovery. While Wilson et al. (2013b) had reported positive effects of a short-term HMB-FA supplementation on markers of exercise-induced muscle damage (EIMD), Gonzalez et al. (2014b) had reported no effects of HMB-FA supplementation on muscle power, strength and endurance. Therefore, although HMB-FA may attenuate inflammatory responses and improve anabolic hormones release (Townsend et al. 2013; Gonzalez et al. 2014a; Townsend et al. 2015), the effects of its supplementation on muscle performance and recovery are still unclear. Furthermore, a number of issues have been raised considering several publications related to HMB-FA (Gentles and Phillips 2017), and thus additional independent studies are clearly needed.

Several divergent supplementation protocols have been applied to study the effect of HMB-FA. While Wilson et al. (2013b) have applied two days with 3g/day of HMB-FA divided equally into three servings, Gonzalez et al. (2014b), Gonzalez et al. (2014a), and Townsend et al. (2013) have applied three days with 3g/day of HMB-FA divided equally into three servings. Recently, Townsend et al. (2015) have investigated the effects of a single-dose of 1g of HMB-FA 30 minutes prior to the exercise protocol, and observed augmented growth hormone response to high volume exercise. Considering that the majority of the studies have administered 3g/day, and that a single-dose supplementation could simplify the athletes’
routine, it is crucial to understand the effect of a 3g single-dose supplementation of HMB-FA. Therefore, the purpose of this study was to investigate the effects of a single-dose supplementation of HMB-FA on muscle recovery after a high-intensity exercise.

Materials and methods

Participants

Twenty-three trained young males aged between 18 and 30 years took part in the present study. Participants characteristics are presented in Table 1. Volunteers were invited from the university sports facilities and were involved in regular training for at least one year before the study protocol. All volunteers were regularly engaged in strength training, besides competing in amateur sports such as rugby, swimming, cycling, or basketball. Individuals were excluded if they had cardiovascular, respiratory, muscular, metabolic or neuroendocrine disorders. They were also excluded if they had been consuming alcohol, creatine, beta-alanine, caffeine, ephedrine, ornithine, branched chain amino acids, carnitine, leucine or its metabolites, arginine, tryptophan, and/or antioxidants in the previous 30 days before enrollment in the study. Additionally, participants were excluded if they had used anabolic steroids or hormonal precursors in the previous year before the study protocol. All participants were fully informed of the purpose, procedures, and possible risks related to the study, and provided a written informed consent. The study was approved by the University Institutional Ethics Committee and was conducted in accordance with ethical standards.

Experimental design
A randomized, double-blinded, placebo-controlled study was conducted. The experimental design is summarized on Figure 1. Due to the potential influence of repeated exercise bouts on muscle damage levels and time course of recovery, a cross-over design was not applied (Miyama and Nosaka 2007; Vieira et al. 2016). Volunteers were randomly assigned to receive either HMB-FA (n = 12) or placebo (PLA; n = 11). The groups were run in parallel, with each group receiving only one form of treatment. Individuals visited the laboratory on four occasions. On the first visit, clinical and anthropometric measurements were assessed and after that volunteers performed seven sets of 20 drop jumps to induce muscle damage. Indirect markers of EIMD were assessed through four outcome variables as follows: muscle swelling, countermovement jump (CMJ), maximal voluntary isometric torque (MVIT), and work capacity (WC). These markers were measured at baseline (Pre), immediately after the exercise protocol (Post), and 24, 48 and 72 hours after the exercise protocol. Immediately after baseline assessment, volunteers received HMB-FA or PLA and rested for 60 minutes before the exercise protocol. All measurements and protocols were conducted in the morning to avoid circadian variations. Over the study protocol volunteers were instructed to maintain their usual dietary intake.

Twenty-four-hour dietary recall

In order to assess dietary intake throughout the experimental protocol, a 24-hour dietary recall was applied every visit to the laboratory to estimate dietary outcomes, including total energy, lipids, carbohydrates, proteins, and leucine. (Shim et al. 2014). In brief, interviews were conducted in-depth and face to face by a certified sports nutritionist and experienced with the procedures. Participants were asked to record all data regarding food preparation methods, ingredients, beverages, the brand name of commercial products, and
specific time of each meal. The portion of each food consumed were estimated in reference to
standard measuring cups and spoons, as well as common size containers. Data were
subsequently analyzed using the software DietWin Plus® (DietWin, Porto Alegre, Brazil).

**Protocol to induce muscle damage**

The exercise protocol consisted of seven sets of 20 drop jumps from a 60-cm box with
2-min rest intervals between sets. After dropping down from the box and landing on the floor,
participants were instructed to perform a maximally explosive vertical jump upward and then
land on the floor. They were instructed to flex their knees at 90º (0º = full extension) during
all landings and to keep their hands on their hips during the jumps. They were verbally
encouraged to exert maximal effort during all repetitions. Each repetition took, in average,
five seconds, and the full protocol took, in average, 24 minutes. This exercise protocol
requires activation of a large lower-limb muscle mass with an eccentric loading to 90º
followed by a high-intensity ballistic concentric action (Ferreira-Junior et al. 2015; Vieira et
al. 2016).

**HMB-FA and PLA supplementation**

The serving of HMB-FA supplement consisted in 1g of β-hydroxy-β-methylbutyrate
in the free acid form, acquired as the commercial product on food supplement Clear Muscle®
(Muscletech, Oakville, CAN). Of note, the product undergoes a strict quality control and is
independently tested to ensure its purity. The serving of PLA consisted in 1g of polydextrose
and was identical to the HMB-FA supplement in appearance and taste. Both HMB-FA and
PLA contain no calories per serving. Both groups consumed three servings (3g) of HMB-FA
or PLA 60 minutes before the exercise protocol. All HMB-FA and PLA ingestion took place in the Strength Training Research Lab and was witnessed by one of the investigators. The blind process was carried out by a pharmacy (Farmacotécnica, Brasília, DF Brazil). HMB-FA and PLA were differed by codes during the study; the code of each substance was revealed after all experimental and statistical procedures were performed in order to clarify the results.

**Muscle Swelling**

Muscle swelling was measured on the right knee extensors using a B-mode ultrasound (Philips, VMI, Lagoa Santa, Brazil). The measurement of muscle swelling was taken at 60% of the distance from the greater trochanter to the lateral epicondyle and 3 cm lateral to the midline of the anterior thigh (Cadore et al. 2012). Once the examiner found a satisfactory image, it was frozen on the monitor (Bemben 2002), stored and analyzed in software Image-J (National Institute of Health, USA, version 1.49). The distance between subcutaneous adipose tissue-rectus femoris interface and vastus intermedius-bone interface was designated as knee extensors muscle swelling. All measurements and analyses were performed three times by the same researcher and the mean value was considered for analysis. The ultrasound intra-rater reliability was 0.94 and the coefficient of variation was 2.4%. A 7.5-MHz scanning probe was placed on the skin perpendicular to the tissue interface. The scanning probe was coated with a water-soluble transmission gel to provide acoustic contact without depressing the dermal surface. No additional pressure was applied to standardize the compression on the dermal surface. The measurement point was marked with an anechoic tape at baseline to ensure that the same point has been measured in all subsequent assessments.

**Countermovement jump performance**
The CMJ performance is usually measured as an indirect marker of muscle damage in order to represent a functional marker of muscle performance and lower limbs power (Vieira et al. 2015). CMJ was calculated by the time of flight, measured on an AMTI force platform (model BP400600-HF-2000; Advanced Mechanical Technology Inc., Watertown, MA, USA), with a sampling rate of 1000Hz. Data were obtained from an acquisition software (AMTI Acquisition Software, v.42; Advanced Mechanical Technology, Inc., Watertown, MA, USA) during vertical jumps and were processed using MATLAB software (version R2008a7, The MAthWorks Inc., Natick, MA, USA). To perform CMJ, volunteers were asked to keep their hands on their hips, start the movement on the standing position, flex knees and hip, and jump as high as possible. A self-determined range of motion was permitted and they received verbal encouragement by the same researcher. Subjects performed five CMJ (two as warm-up and three attempts to achieve their best jump performance). Between all jumps volunteers rested for 60 seconds. The greatest jump height was considered as CMJ performance and was used for further analyses. Jump height was calculated by the flight duration (fd) according the following equation: jump height = (fd<sup>2</sup> x 9.81)/8. All measurements and analyses were conducted by the same researcher. The countermovement jump intra-rater reliability was 0.93 and the coefficient of variation was 4.1%.

**Maximal voluntary isometric torque and work capacity**

MVIT and WC were measured using an isokinetic dynamometer Biodex System 3 (Biodex Medical Systems, Inc., New York, USA). The volunteers were positioned on the dynamometer seat with velcro belts fastened to the trunk, pelvis and thigh to avoid extraneous body movements that could affect results. The lateral epicondyle of the femur was used to
align the knee rotation axis and the dynamometer rotation axis, allowing free knee extension and flexion from 85° flexion up to full extension. Gravity correction was obtained by measuring the torque exerted by the lever arm and the subject’s leg at 30° flexion as well as in a relaxed position. The values of the isokinetic variables were automatically adjusted for gravity with the software Biodex Advantage (Biodex Medical Systems, Inc., New York, USA). Calibration of the dynamometer was carried out according to the manufacturer specifications. For the test, volunteers were asked to cross their arms across the chest. The same researcher carried out the procedures for all subjects and provided verbal encouragement. MVIT was measured on right knee extensors at 60° (0° = full extension) on the dominant leg. The volunteers had two attempts of four seconds to achieve their maximal isometric torque with 1-min rest intervals between attempts. WC was also measured on the right knee extensors Three minutes after MVIT protocol, volunteers performed 30 maximal isokinetic knee extensions at 120°/s for WC evaluation. Work capacity was calculated as the amount of torque produced throughout the entire range of motion of all repetitions. Test-retest reliability coefficient (ICC) value for knee extensor peak torque was 0.91 in our laboratory.

**Statistical analyses**

Data are presented as mean and standard deviation. Normal distribution parameters were checked with Shapiro-Wilk test. Physical characteristics and training status were compared between groups by an independent t test. Dietary intake outcomes (total energy intake, carbohydrate, protein, lipids, and leucine), muscle swelling, CMJ performance, MVIT and WC were analyzed by a mixed-model analyses of variance with two factors (group x time). The Bonferroni adjustment was applied as post hoc analysis if any interaction was found. The effect size was calculated and reported ($\eta_p^2$). The Statistical Package for Social
Sciences (SPSS), version 21.0 (IBM, USA) was used for all analyses. The alpha level was set at 5% (p ≤ 0.05). Retrospective statistical power (1-β) was calculated by the G*Power software (version 3.1.9.2).

**Results**

There were no between-group differences (p > 0.05) for physical characteristics and training status. Moreover, there were no between-group differences (p > 0.05) for knee extensors thickness, CMJ, MVIT and WC on baseline. There were no within-group or between-group differences (p > 0.05) for total energy intake, carbohydrates, proteins, lipids, and leucine at any time-point.

Muscle swelling, CMJ and MVIT on both groups and all time-points are reported on table 2. Regarding muscle swelling, there was a significant main effect for time (F = 57.496; p < 0.001; $\eta_p^2 = 0.732$), but not for group (F = 0.040; p = 0.947; $\eta_p^2 = 0.000$). Also, there was no significant group by time interaction (F = 0.433; p = 0.716; $\eta_p^2 = 0.020$). Muscle swelling increased similarly on both groups after the exercise protocol (p < 0.001), and returned to pre-exercise levels 24 hours after the exercise protocol. Regarding CMJ, there was also a significant main effect for time (F = 10.140; p < 0.001; $\eta_p^2 = 0.326$), but not for group (F = 0.016; p = 0.901; $\eta_p^2 = 0.001$). Similarly, there was no significant group by time interaction (F = 0.476; p = 0.588; $\eta_p^2 = 0.022$). CMJ decreased similarly on both groups after the exercise protocol (p < 0.05), and returned to pre-exercise levels after 24 hours. On MVIT, there was also a significant main effect for time (F = 24.334; p < 0.001; $\eta_p^2 = 0.537$), but not for group (F = 2.142; p = 0.158; $\eta_p^2 = 0.093$) nor group by time interaction (F = 0.254; p = 0.814; $\eta_p^2 = 0.012$). MVIT decreased similarly on both groups after exercise protocol (p < 0.001), and returned to pre-exercise levels after 24 hours.
Regarding WC (Figure 2), there was a significant main effect for time \( (F = 23.257; \ p < 0.001; \ \eta_p^2 = 0.526) \) and for group \( (F = 3.906; \ p = 0.041; \ \eta_p^2 = 0.157) \). There was also a significant group by time interaction \( (F = 3.979; \ p = 0.019; \ \eta_p^2 = 0.159; \ \text{power (1-} \beta) = 0.88) \). WC decreased similarly on both groups after the exercise protocol \( (p < 0.01) \). However, on HMB-FA, WC returned to pre-exercise level 24 hours after exercise protocol. For PLA, WC did not return to pre-exercise level even 72 hours after the exercise protocol. Consequently, WC was significantly lower on PLA compared to HMB-FA 24 hours \( (p = 0.039) \), 48 hours \( (p = 0.021) \), and 72 hours \( (p = 0.048) \) after exercise protocol.

**Discussion**

To the best of our knowledge, no previous studies have explored the acute effects of a single-dose HMB-FA supplementation on muscle recovery after an exercise session. This randomized, double-blinded, placebo-controlled trial aimed to investigate the effect of a single-dose of HMB-FA supplementation on muscle recovery after a high-intensity exercise bout. The salient findings of the present investigation suggested that a single-dose of HMB-FA supplementation improves work capacity recovery in trained young men. No significant HMB-FA effects were noted for muscle swelling, muscle strength, and muscle performance after a high-intensity exercise. These results provide support for the concept that HMB-FA supplementation may improve recovery after intense exercise (Silva et al. 2017). From a practical standpoint, HMB-FA could be consumed to improve work capacity recovery in trained individuals enrolled in high-intensity short-term activities. Nevertheless, more studies are needed to determine the overall efficacy of HMB-FA supplementation as an ergogenic aid.
This is the first study examining the effects of a single-dose HMB-FA supplementation on muscle swelling. This outcome is usually used as an indirect marker of exercise-induced muscle damage as result of an inflammatory response induced by the disruption of myofibrils during high-intensity eccentric exercise (Paulsen et al. 2012). Muscle swelling is also related to metabolic stress and may represent acute alterations in metabolic pathways, pH, and energy production (Radaelli et al. 2012). Although previous studies have reported that HMB-FA could attenuate inflammatory responses induced by an acute bout of high-intensity exercise (Townsend et al. 2013; Wilson et al. 2013b; Gonzalez et al. 2014a; Gonzalez et al. 2014b), the present study showed no differences on muscle swelling between HMB-FA and PLA. Such absence of difference could be related to the training status of subjects, as evidenced by muscle swelling recovery 24 hours after exercise protocol. According to Newton et al. (2008), trained subjects exhibit less evident changes in muscle function when compared to their untrained counterparts after an eccentric exercise due to an attenuated inflammatory response. Therefore, the effects of the used muscle damage protocol on muscle swelling could be attenuated in trained subjects due to a protective effect against inflammation and protein breakdown (Holecek 2017). Consistent with this assumption, both HMB-FA and PLA muscle swelling returned to baseline levels 24hs after the protocol.

Similarly, HMB-FA had no effect on MVIT and CMJ compared to PLA. Muscle strength, such as isometric peak torque, is one of the most common methods used to assess muscle recovery (Warren et al. 1999; Paulsen et al. 2012). In addition, CMJ represent a functional measurement of muscle performance given the fact that it requires coordination of several neuromuscular mechanisms, such as storage and reutilization of elastic energy, and potentiation of contractile machinery (Byrne et al. 2004; Vieira et al. 2016). Only one study has investigated the effect of an acute supplementation of HMB-FA on muscle performance (Gonzalez et al. 2014b). The findings of the abovementioned report showed no effect of
HMB-FA on muscle performance recovery, with muscle strength and power remaining lower than pre-exercise levels even 48 hours after the exercise protocol. However, in the present study, MVIT and CMJ returned to pre-exercise levels 24 hours after the high intensity exercise bout even in PLA. Such phenomenon could be also related to volunteers’ training status, since trained subjects develop a protective effect from training movements and loads, which has been referred to as repeated bout effect (Brandenburg and Docherty 2002; Brentano and Kruehl 2011). Therefore, trained subjects could be less susceptible to decreases in muscle performance and exercise-induced muscle damage could be dependent, at least partially, on training experience (Paschalis et al. 2005; Newton et al. 2008; Lima and Denadai 2015; Ye et al. 2015).

In the present study, a single-dose supplementation of HMB-FA improved the recovery of WC compared to PLA. Different from muscle swelling, CMJ or MVIT, WC did not return to pre-exercise levels even 72 hours after the exercise protocol in PLA, while subjects exposed HMB-FA acute supplementation have totally restored their WC 24 hours after the exercise protocol. Interestingly, previous studies have reported the dissociation between the time-course of peak torque and WC recovery in trained subjects (Ferreira et al. 2017). Such phenomenon occurs because muscle strength and muscle fatigability have different related mechanisms. During a high-intensity exercise, a complex sequence of events occurs, including sarcomere disruption, increased inflammatory response, depletion of muscle glycogen, decreased insulin sensitivity, and reduction of glucose transporters’ mobilization (Asp et al. 1996; Proske and Morgan 2001; Tee et al. 2007; Ferreira et al. 2017). This cascade of events could impair metabolic function and decrease WC for several days after a single high-intensity exercise bout (Peake et al. 2005; Hughes et al. 2013). In this regard, several studies have showed the effectiveness of HMB-FA to attenuate inflammatory responses after a high-intensity exercise (Townsend et al. 2013; Wilson et al. 2013b; Gonzalez et al. 2014a;
Moreover, the acute supplementation of HMB-FA seems to improve protein synthesis and inhibit protein breakdown, accelerating the regeneration of sarcomere membrane (Wilkinson et al. 2013; Wilson et al. 2013b). Such pathways may contribute to restore muscle glycogen and recover WC after a high-intensity exercise, suggesting that a single-dose supplementation of HMB-FA could be taken to improve recovery by athletes enrolled in high intensity activities with duration between 20 and 120 seconds (Ferreira et al. 2017).

The current study has several strengths and limitations. The randomized, double-bind, placebo-controlled design, and novelty of the results are strengths. A limitation was that direct measurement of inflammatory markers, metabolic stress, and protein turnover were not included. Nevertheless, it has been argued that changes in muscle function is the best marker for the degree of exercise-induced muscle damage (Paulsen et al. 2012). Another limitation was the inclusion exclusively of trained men. It has been suggested HMB-FA affects trained and untrained subjects in different ways (Holecek 2017), and thus the results may not be applicable to untrained subjects. Lastly, the lack of a standard dietary prescription might have allowed some variation in nutrient intake distribution. However, subjects were systematically instructed to maintain their habitual dietary intake and the 24-hours dietary recall were applied in each individual in order to minimize this shortcoming, with no differences within or between groups observed.

In summary, a single-dose supplementation of β-hydroxy-β-methylbutyrate free acid improved the time-course of WC recovery after a high-intensity exercise bout. The supplementation protocol did not affect the time-course of muscle swelling, MVIT and CMJ performance recovery. Future studies should confirm these findings and examine the use of HMB-FA as a supplement to improve performance recovery in practical settings in individuals enrolled in high-intensity activities.
Conflict of Interest Disclaimer

The authors report no conflicts of interest associated with this manuscript.

Acknowledgments

The authors would like to thank the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES, University of Brasilia, and Brazilian National Council for Scientific and Technological Development (CNPq).

References


https://mc06.manuscriptcentral.com/apnm-pubs


### Tables

#### Table 1 Physical characteristics and training status of studied individuals.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HMB-FA (n = 12)</th>
<th>PLA (n = 11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.75 ± 2.96</td>
<td>22.82 ± 3.09</td>
<td>0.957</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81.17 ± 6.29</td>
<td>76.30 ± 7.28</td>
<td>0.100</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.82 ± 0.04</td>
<td>1.79 ± 0.02</td>
<td>0.085</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.52 ± 1.98</td>
<td>23.85 ± 2.14</td>
<td>0.447</td>
</tr>
<tr>
<td>Knee extensor thickness (mm)</td>
<td>41.41 ± 5.41</td>
<td>41.25 ± 6.45</td>
<td>0.950</td>
</tr>
<tr>
<td>CMJ (cm)</td>
<td>40.64 ± 7.51</td>
<td>42.30 ± 4.86</td>
<td>0.539</td>
</tr>
<tr>
<td>Peak torque (N.m)</td>
<td>319.55 ± 37.97</td>
<td>293.97 ± 47.87</td>
<td>0.169</td>
</tr>
<tr>
<td>Work capacity (J)</td>
<td>5378.66 ± 366.57</td>
<td>5346.75 ± 525.79</td>
<td>0.867</td>
</tr>
<tr>
<td>Training experience (years)</td>
<td>3.38 ± 1.49</td>
<td>3.86 ± 2.77</td>
<td>0.600</td>
</tr>
<tr>
<td>Training frequency (days/week)</td>
<td>4.33 ± 0.98</td>
<td>4.91 ± 0.94</td>
<td>0.168</td>
</tr>
<tr>
<td>Training duration (min/day)</td>
<td>62.08 ± 18.52</td>
<td>59.09 ± 17.72</td>
<td>0.697</td>
</tr>
</tbody>
</table>

BMI: body mass index; CMJ: countermovement jump
Table 2 Muscle swelling, CMJ and MVIT on both groups and all time-points. Data are presented as mean ± standard deviation.

<table>
<thead>
<tr>
<th>Variables (mean ± SD)</th>
<th>HMB-FA (n = 12)</th>
<th>PLA (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muscle swelling (mm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>41.41 ± 5.41</td>
<td>41.25 ± 6.45</td>
</tr>
<tr>
<td>Post</td>
<td>49.41 ± 6.21*</td>
<td>48.52 ± 8.50*</td>
</tr>
<tr>
<td>24 hours</td>
<td>42.48 ± 5.61</td>
<td>43.06 ± 7.25</td>
</tr>
<tr>
<td>48 hours</td>
<td>42.79 ± 5.28</td>
<td>42.53 ± 6.20</td>
</tr>
<tr>
<td>72 hours</td>
<td>42.56 ± 4.86</td>
<td>42.45 ± 6.53</td>
</tr>
<tr>
<td><strong>CMJ (cm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>40.64 ± 7.51</td>
<td>42.30 ± 4.86</td>
</tr>
<tr>
<td>Post</td>
<td>36.60 ± 8.87*</td>
<td>36.91 ± 4.27*</td>
</tr>
<tr>
<td>24 hours</td>
<td>39.35 ± 6.72</td>
<td>39.45 ± 4.24</td>
</tr>
<tr>
<td>48 hours</td>
<td>39.85 ± 7.21</td>
<td>39.80 ± 4.69</td>
</tr>
<tr>
<td>72 hours</td>
<td>41.52 ± 7.14</td>
<td>41.00 ± 4.22</td>
</tr>
<tr>
<td><strong>MVIT (N.m)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>319.55 ± 37.97</td>
<td>293.97 ± 47.87</td>
</tr>
<tr>
<td>Post</td>
<td>254.66 ± 59.89*</td>
<td>232.42 ± 39.03*</td>
</tr>
<tr>
<td>24 hours</td>
<td>300.43 ± 38.71</td>
<td>266.60 ± 53.02</td>
</tr>
<tr>
<td>48 hours</td>
<td>305.14 ± 39.28</td>
<td>282.94 ± 65.67</td>
</tr>
<tr>
<td>72 hours</td>
<td>310.37 ± 39.54</td>
<td>280.89 ± 56.60</td>
</tr>
</tbody>
</table>

CMJ: countermovement jump; MVIT: maximal voluntary isometric torque.

*p ≤ 0.05, significantly different from pre-exercise values.
Figure Captions

Figure 1 Experimental design and time-points of indirect markers of muscle damage measurements. CMJ: countermovement jump; MVIT: maximal voluntary isometric torque; WC: work capacity.

Figure 2 Work capacity on both groups across the 72 hours of experimental protocol (mean ± SD). * p ≤ 0.05, significantly different from pre-exercise values; # p ≤ 0.05, significantly different from HMB-FA group.
Muscle Swelling
CMJ
MVIT
WC

HMB-FA or placebo

Protocol to induce muscle damage

60 min | 24 hours | 24 hours | 24 hours
The diagram illustrates the change in WC (J) over time for two groups: HMB-FA and PLA. The x-axis represents time points (Pre, Post, 24h, 48h, 72h), while the y-axis represents the WC (J) with a range from 2000 to 7000. The HMB-FA group shows a decrease in WC from Pre to Post, with a return to baseline by 24h. The PLA group remains relatively stable throughout the time points. Significant differences are indicated by asterisks (* for HMB-FA vs PLA) and hash marks (# for specific time points within each group).