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The Effects of Supplementation of β -Hydroxy- β -Methylbutyrate on Inflammatory Markers in High Performance Athletes

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ABSTRACT

Ferreira HF, Rodacki ALF, Gill P, Tanhoffer R, Fernandes Filho J, Fernandes LC. The Effects of Supplementation of β -Hydroxy- β -Methylbutyrate on Inflammatory Markers in High Performance Athletes. *JEPonline* 2013;16(1):53-63. The use of ergogenic nutritional supplements is becoming inseparable from competitive sports. The leucine metabolite β -hydroxy- β -methylbutyrate (HMB) has been used as an ergogenic aid to promote increased fat-free mass (FFM) and strength gains during resistance training. The purpose of this randomized, double-blind, placebo-controlled study was to determine the effects of $37.5 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ HMB supplementation on body composition, sports performance, and inflammatory mediators. The subjects consisted of 20 elite paddlers, during strength training for 6 months. There was an increase in lean body mass (2.7 kg $P < 0.05$), reduced levels of CK and LDH (-37.23 and $-20.92 \text{ U}\cdot\text{L}^{-1}$, $P < 0.05$, respectively), and a decrease in time official race of -4.45 sec in the HMB group. The results indicate that the proposed dosage of HMB can increase lean body mass and strength in association with resistance training in already trained athletes. The mechanism by which this occurs is still unknown. Also, the findings indicate that there may be a decrease in the damage to skeletal muscle when stressed before training.

Key Words: Paddlers. HMB, Ergogenic Supplements, Canoeist

INTRODUCTION

It is believed that athletes use the leucine metabolite β -hydroxy- β -methylbutyrate (HMB) to promote skeletal muscle mass to increase athletic performance. As an ergogenic aid, it is used by strength and power athletes, bodybuilders, and athletes in high performance sports. There is also evidence to suggest that HMB plays a role in specific physiological and biochemical variables in relation to metabolic demands during athletic competitions. Although the role is a positive one in that it appears to increase the athletes' chances of achieving success, it is important to point out that benefits from HMB may not be the same for all types of athletes. Naturally, when HMB works well with training, it encourages coaches and athletes in the pursuit of gains in athletic performance. They are more prone to use various nutritional strategies and/or dietary supplements (7).

Although not clearly understood, it has been demonstrated that leucine has anti-catabolic properties (27). This means that the metabolite of leucine, α -ketoisocaproate (KIC) appears to contribute to these results (26) by decreasing muscle breakdown. Thus, the ingestion of dietary supplement containing proteins may promote an increase in the rate of total body protein synthesis and suppression of protein degradation (2,8,11). This anabolic effect can be attributed to the increased contribution of amino acids to skeletal muscle. In particular, leucine, a branched chain amino acid, has the ability to independently stimulate protein synthesis (1) and thus as a regulator of protein metabolism (20).

There is evidence that untrained men and women (22) who are involved in 3 to 4-wk resistance training program show a higher gain in fat-free mass (FFM) and strength with doses of 1.5 to 3 g·d⁻¹ of HMB. The FFM gain is associated with a significant loss in the excretion of 3-methylhistidine muscle, suggesting a decrease in catabolism (22). In agreement, after elderly men and women engaged in a beginner training program for 8 wks with supplementation of 3 g·d⁻¹ of HMB, there was a significant increase in lean body mass and in 1RM strength, and a decrease in fat mass (37). Gallagher and colleagues (10) reported on the effects of HMB supplementation of 0.38 and 0.76 mg·kg⁻¹·d⁻¹ during 8 wks of resistance training in untrained men. They found a significant decrease in the excretion of creatine kinase and increased lean body mass only in the group of 0.38 mg·kg⁻¹·d⁻¹. The results suggest that supplementation with 1.5 to 3 g·d⁻¹ of HMB can increase the FFM and strength in untrained individuals in a resistance training program (22,28,36).

Vukovich and Adams (37) reported that after 2 wks of HMB supplementation (3 g·d⁻¹), there was a significant increase in rate fatigue, lactate threshold, and VO₂ peak in trained cyclists. It seems clear that HMB supplementation provides an ergogenic value during intense exercise (29,38). However, it is clear that further investigations are needed before definitive conclusions can be made regarding the HMB supplementation in all athletes. The purpose of this study was to determine whether HMB supplementation at 37.5 mg·kg⁻¹·d⁻¹ during intense endurance training affects markers of catabolism, body composition, and sports performance in kayak athletes' high performance sprints.

METHODS

Subjects

Twenty elite canoeists volunteered to participate in this study, which took place during their strength training season. The subjects were informed about the procedures and, then, they signed the consent form approved by the ethics committee of the Federal University of Parana (No. 1179.104.11.08). All subjects signed a statement indicating that they never used anabolic/androgenic steroids according to the criteria of the World Anti-Doping Agency (WADA). The subjects were informed that they could

undergo random doping tests in accordance with the need of the Committee Brazilian Olympic (COB) and/or the Brazilian Canoe Confederation (CBCa). Moreover, no subject (i.e., athlete) had a history of a positive test for the presence of anabolic/androgenic steroids.

Procedures

The individual kayak (K1) boat built of carbon fiber (Nelo Vanquish® Model II) was selected. The variables time and speed were verified in the sports performance using fixed distance for the 1,000 m sprint. This test consisted of a single maximum sprint on flat water (lake) that was marked with buoys. The test was held monthly for 6 months at the same climatic conditions.

A stopwatch (Nielsen-Kellerman Interval® 2000®) with precision .001 secs was used to determine the time. After each sprint, samples of venous blood were obtained by venipuncture of the antecubital vein using standard procedures (9,14,33,34). The venous blood was collected in vacuum tubes of 10 ml for serum separation. The tubes were centrifuged at 5000 RPM for 10 min using a bench top refrigerated centrifuge (Excelsa® Model 280R4). Biochemical parameters [Lactate Dehydrogenase (LDH), Creatine Kinase (CK), MB and MM isoenzyme of CK (CKMB and CKMM) and Lipids (LDL, HDL and Triglycerides)] were analyzed using kits of Vital Scientific® (Ellitech Group Spankeren, Netherlands) in the semi-automatic analyzer model 300 MicroLab® Vital Scientific (Ellitech Group, Spankeren, Netherlands). Body weight was determined using a digital scale (Sanny) with an accuracy of ± 0.02 kg (Sterling Scale Co., Southfield, MI). Measurements of body composition (fat mass%) were determined using bioimpedance (35) tetrapolar model BF 906 Maltron ® following the procedures described by the supplier (Maltron ®) (15).

Experimental Approach to the Problem

All subjects maintained the training program during the study. They were randomized and double-blind into the placebo-controlled (PLB) group ($n = 6$) and the “treatment” group supplemented with HMB ($n = 14$). The treatment group received a dose of $37.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ of HMB. The placebo (PLB) group was supplemented with a placebo. All meals were kept under strict control by the nutritionist. None of the subjects was allowed the use of creatine and/or beta-agonists for at least 8 wks prior to the study. All subjects (i.e., athletes) were instructed to not ingest other dietary supplements with ergogenic effects during the study.

The supplements were prepared in powder form by the laboratory Metabolic Technologies, Inc., (Iowa - USA), and were weighted according to body weight for each subject and encapsulated in flasks and conditioned at a specialized laboratory (Botica Ouro Preto – Brazil). All subjects ingested the daily portions, which were divided into three parts (morning, afternoon, and evening). Each vial contained enough supplement for 15 d. Daily control of the supplement was supervised by a coach who was responsible for returning the empty vials and receiving a new supply every 15 d.

The subjects' training sessions were individualized workloads that averaged of $6 \text{ hr} \cdot \text{wk}^{-1}$ of resistance training (1 to 3 sets of 2 to 8 repetitions at intensities ranging from 80 to 95% of 1 RM) on Monday, Wednesday, and Friday plus $10 \text{ hr} \cdot \text{wk}^{-1}$ of specific sprint technique training in the boat. The subjects averaged two daily sessions for a total of 11 training sessions a week. All trainings sessions were conducted under the supervision of certified coaches by CBCa. Subjects who missed a training session were required to make them up in accordance with the procedures of the study. All subjects underwent monthly testing of venous blood samples, body composition analysis, and specific testing for performance in the water (boat).

Statistical Analyses

The analysis of data normality was confirmed by the Wilcoxon test. The comparison of means was carried out using the t test for paired samples (SPSS Inc., Chicago, IL). The delta of change along with the magnitude (pre and post) was calculated with the variables selected and analyzed by ANOVA. Results were expressed as mean \pm standard deviation. The data were considered significantly different when the probability of error was $P < 0.05$.

RESULTS

In relation to the body composition data (Body Weight and % Fat mass) the HMB group showed a decrease in both components (Table 1), unlike the PLB group (Table 2). Also, in relation to body composition over the period studied, there were significant differences between groups (Figure 1). When compared with the biochemical changes, the HMB group (Table 1) demonstrated a significant increase in Serum Creatinine versus the PLB group (Table 2). An interesting finding was that there was a significant decrease in serum CK-MM for the HMB group while there was a significant increase in the PLB group.

In Figure 1, it can be observed that the behavior of the variables (Body Weight, % Fat Mass, Serum Creatinine, and Time - Official Race) decreased in the HMB group. Among the variables of Figure 1, Serum Creatinine in the HMB group was significantly different from the PLB group only at the 1st month ($P < 0.05$). Serum Creatinine showed an interesting behavior over time. There was an initial increase in both groups and, then, at the 2nd and subsequent months, it increased in the PLB group while it decreased in the HMB group.

Figure 2 indicates that there was a marked decrease in all the variables (CK-MM, CK-MB, LDH, and CK Total) with the HMB group demonstrating the greatest decrease across time. Significant differences for CK-MM, CK-MB, and Total CK between the groups occurred at the 2nd month ($P < 0.01$). Also, an interesting finding was that at the 1st month, there was an increase in CK-MM and CK Total for both groups.

Table 1. Pre- and Post-Treatment Biochemical Parameters in the HMB Group for 6 Months.

	HMB Group		
	Pre	Post	M (%)
Body Weight (kg)	78.50 \pm 3.04	76.30 \pm 2.82	-2.80
% Fat Mass (%)	11.11 \pm 2.17	9.97 \pm 1.44	-10.26
Triglycerides (mg·dL⁻¹)	85.50 \pm 22.73	76.55 \pm 8.21	-10.46
LDL Cholesterol(mg·dL⁻¹)	167.20 \pm 37.16	151.30 \pm 26.81	-9.50
HDL Cholesterol(mg·dL⁻¹)	47.10 \pm 4.87	44.40 \pm 4.44	-5.73
Serum Creatinine (mg·dL⁻¹)	0.84 \pm 0.15	1.00 \pm 0.08*	+16.00
Total Serum CK (U·L⁻¹)	299.95 \pm 27.47	188.25 \pm 24.32	-37.23
Serum CK-MM (U·L⁻¹)	192.76 \pm 26.95	126.36 \pm 28.81*	-34.44
Serum CK-MB (U·L⁻¹)	122.25 \pm 28.51	65.88 \pm 15.51	-46.11
Lactate Dehydrogenase (U·L⁻¹)	367.25 \pm 30.25	290.40 \pm 26.34	-20.92
Official Time - Race (s)	202.40 \pm 4.89	197.95 \pm 5.23	-2.19

Where: M =% magnitude (-) decrease, (+) increase. Mean \pm SD, * $P < 0.05$.

Table 2. Pre- and Post-Treatment Biochemical Parameters in the PLB Group for 6 Months.

PLB Group			
	Pre	Post	M (%)
Body Weight (kg)	79.38 ± 3.56	78.70 ± 3.46	-0.86
% Fat Mass (%)	9.63 ± 1.62	9.65 ± 1.62	+0.21
Triglycerides (mg·dL⁻¹)	86.10 ± 20.76	78.66 ± 11.48	-8.64
LDL Cholesterol (mg·dL⁻¹)	148.90 ± 22.61	156.70 ± 29.62	+5.24
HDL Cholesterol (mg·dL⁻¹)	39.90 ± 5.02	40.90 ± 2.88	+2.51
Serum Creatinine (mg·dL⁻¹)	0.94 ± 0.15	1.02 ± 0.06*	+7.84
Total Serum CK (U·L⁻¹)	263.20 ± 52.28	268.5 ± 31.00	+2.01
Serum CK-MM (U·L⁻¹)	124.28 ± 33.98	174.52 ± 20.15*	+40.42
Serum CK-MB (U·L⁻¹)	66.92 ± 18.29	93.97 ± 10.85	+39.07
Lactate Dehydrogenase ((U·L⁻¹)	336.60 ± 53.30	332.90 ± 50.85	-1.01
Official Time - Race (s)	204.84 ± 4.38	202.71 ± 5.32	-1.04

Where: M =% magnitude (-) decrease, (+) increase. Mean ± SD, *P<0.05.

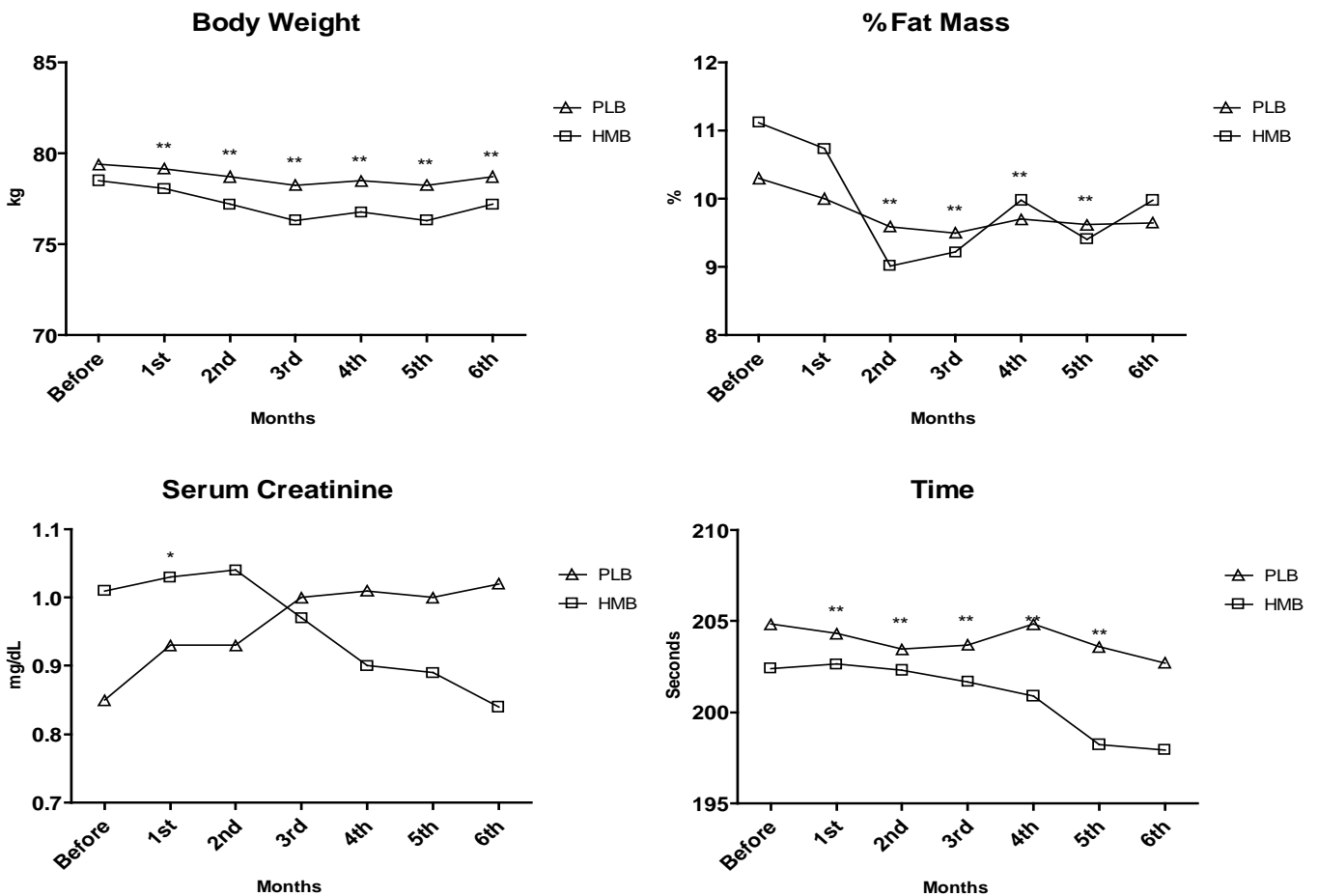


Figure 1. Values of Body Weight (kg), Fat Mass (%), Serum Creatinine (mg·dL⁻¹), and Race Official Time (sec) over the 6 months for the HMB and PLB groups (Mean ± SD). *P<0.05, **P<0.01

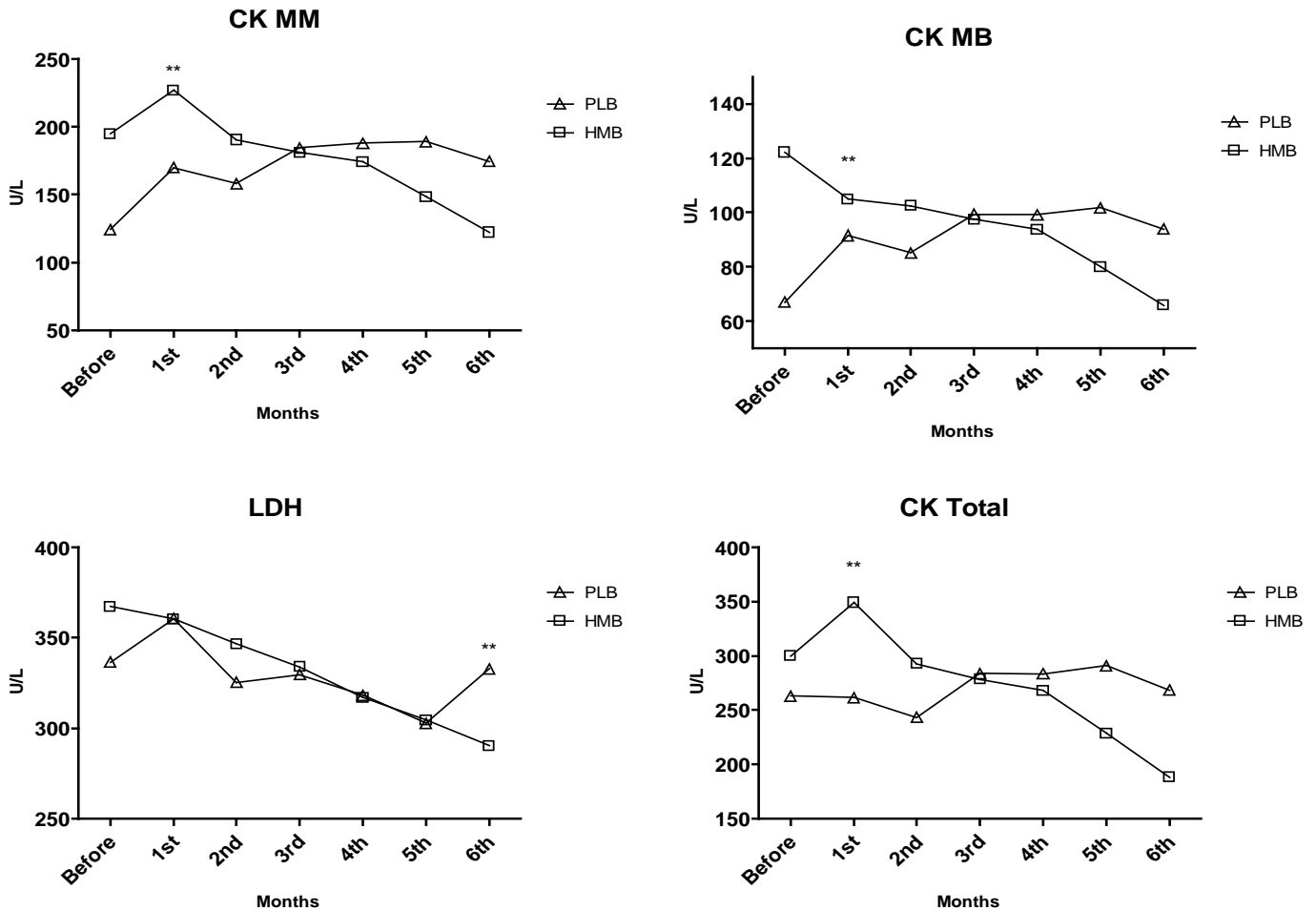


Figure 2. Values of CK-MM ($\text{U}\cdot\text{L}^{-1}$), CK-MB ($\text{U}\cdot\text{L}^{-1}$), Lactate Dehydrogenase ($\text{U}\cdot\text{L}^{-1}$), and CK Total ($\text{U}\cdot\text{L}^{-1}$) over the 6 months for the HMB and PLB groups (Mean \pm SD). ** $P<0.01$

DISCUSSION

This study identified a significant reduction in the triglycerides, HDL, and LDL in both the HMB group and the PLB group after 6 months of the same training sessions. This confirms the findings in the international literature (21) and in the hypothesis about the change in lipid parameters (30).

Nissen and colleagues (22) reported that supplementation with HMB ($3\text{ g}\cdot\text{d}^{-1}$) resulted in a significant change in muscle function with respect to strength and body composition (3.10% increase in lean mass, $P<0.01$; -7.3% reduction of fat mass, $P<0.03$) in response to intense resistance training. Portal and colleagues (31) found a significant increase in lean body mass in elite volleyball athletes when supplemented with HMB ($3\text{ g}\cdot\text{d}^{-1}$) for 7 wks (31). Whereas the strength in trained athletes have less potential for an increase when compared to untrained athletes (12). Others studies (16-18,21-23) show that athletes supplemented with HMB ($3\text{ g}\cdot\text{d}^{-1}$) during 7 wks of training resulted in a significant increase in lean mass (approximately 2.7 kg, $P<0.05$), thus confirming the data found in this study. Athletes supplemented with HMB lost 2.2 kg of body weight, which was the case with the PLB group as well, but the difference was not significant.

Although only a few studies were found a gain strength in subjects supplemented with HMB, it is also believed that the increase in lean body mass is important for many sports (7), among them canoeing sprint (19). The canoeing sprint and football athletes required a large accumulation of energy reserves, thus the volume of lean body mass is crucial (22). In particular, in the canoeing race of K1-1000 m, there are many moments characterized by increased intensity of muscle contraction and speed (requiring a greater recruitment of type II muscle fibers). The increased lean body mass volume allows for an increase in the reserve mobilization of phosphocreatine for the regeneration of ATP, resulting in an increase in serum creatinine (5,13,24) that supports the findings in the present study for both groups (HMB and PLB).

It is known that high intensity training may require excessive force, this may cause an overload in the muscles and as well in the contractile systems with structural disruption (6,9,14,33,34). This generates higher neutrophil infiltration; consequently, the release of cellular proteins (e.g., CK). The increased activity of plasma muscle enzymes such as LDH, CK, and respective fractions may be a physiological response typical of very strenuous exercise. As a result, these enzymes can be used as markers of muscle damage. In the present study, there was a decrease in LDH in both groups (HMB and PLB). However, the decrease was more pronounced for HMB group (-20.92%), which is in agreement with the literature (22,32).

The activity of enzymes LDH and CK are considered important markers of muscle damage, however, are values isolated, because are parameters indirect. Furthermore, there are variations in activity according to the conditions of volume and intensity of training (4). The PLB group in this study demonstrated an increase in CK, however not significant and HMB group had a significant reduction in LDH (-16%, $P < 0.05$) (23).

The muscle damage by exercise is characterized by decreased force, increased inflammation, breakdown of muscle fibers and increased activity of proteolytic enzymes. The elevation of CK may be associated with an adaptive microtrauma in that highly trained athletes may be a constant response, able to accelerate the turnover of muscle fibers. If the workload is repeated over time, this muscle damage is reduced and the athlete develops an adaptation in skeletal muscles, characterized by a reduction in the release of CK. This may explain the drop in levels of total CK from the fourth month of training in this study (25). About concentrations CK total, in this study, there was a reduction, not significant in group HMB corroborates findings in the literature (23). On other way, in the group PLB there was a slight increase (+2.01%), but not significant.

The results of the activity of CK-MM showed an increase for the PLB group ($P = .04$) for this release bloodstream is more specific to muscle overload when compared to total CK (4,6,14,34). The HMB group showed a significant decrease ($P = .04$) progressive over months again suggesting a protective effect of HMB supplementation versus catabolism imposed by resistance exercise training, contrary to the PLB group (36).

Numerous hypotheses have been established in order to explain the adaptive micro injuries, among some assumes the occurrence of a metabolic overload in which the need for ATP would become higher than its rate of production, another theory proposes that muscle injury may be caused by mechanical forces, such as the eccentric contraction, able to break the muscle architecture; another hypothesis is the increase of inflammation mediators and oxidative stress (4,6,14,34). Concentrations isoenzyme MB of Creatine Kinase (CK-MB) shows the same behavior that Isoenzyme MM of Creatine Kinase (CK-MM) for both groups. The increase in serum CK-MB can happen due to atypical shape CK, for example, macro-CK, which is a complex consisting of CK-BB bound to immunoglobulin (IgA, IgG), whose presence can causing an apparent increase in the activity of CK-MB.

In canoeing, the main index is the race time (3,19) where the difference between the final contestants in an international championship is close to 1 sec. We observed that during the present study there was no significant reduction ($P=.11$) of 4.45 sec (-2.19%) for the HMB group and of 2.13 sec (-1.04%) for the PLB group, although perhaps relevant (34). The response cannot be considered an improvement due to HMB supplementation, however biochemical parameters and body composition appears to suggest such. The data show improvement of approximately 4 sec on a boat Olympic, that is very significant when compared on the international scene. The present results regarding HMB supplementation, along with other recent studies of sports medicine have established opportunities for improvement in quality of life, as well as in the athletic performance in sports. Recommend that future research explores supplementation in different dosages and different populations, as well as injury recovery.

CONCLUSIONS

The results of this study indicate that supplementation with HMB at $37.5 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$, can increase lean body mass, strength gains associated with resistance training in already trained athletes of high performance. The correct mechanism by which this can occur is still unknown, but these results indicated that there may be a decrease in the skeletal muscle damage. There was a response towards the improvement of sports performance greater for the supplemented group. While research so far are encouraging, obviously there need for further studies controlled and long-lasting to verify, the possible effects in the improvement in strength and development of muscular hypertrophy helping not only athletes but the general population, which has some muscle atrophy and elderly.

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