

Beta-Hydroxy-Beta-Methylbutyrate Supplementation Preserves Fat-Free Mass in Collegiate Boxers during Acute Body Mass Loss

Chen-Kang Chang^{1*}, Shih-Yen Kao¹, Chung-Yuan Wang²

¹Department of Sport Performance, National Taiwan University of Sport, Taichung, Taiwan, ²Department of Combat Sport, National Taiwan University of Sport, Taichung, Taiwan

Abstract

Acute body mass loss before competitions in combat sports usually leads to loss in fat-free mass. Beta-hydroxy-beta-methylbutyrate (HMB) has been shown to increase skeletal muscle mass and muscle strength in various muscle wasting conditions. This study investigated the effect of HMB supplementation on body composition and sport-specific performance in well-trained boxers consuming a hypocaloric diet. Twelve male college boxers were divided into the HMB and placebo (PLA) groups using a body weight-matched single-blind parallel design. The study comprised a 6-day weight loss period (days 1–6), followed by a 3-day competition period (days 7–9). The participants in both the groups consumed 16 kcal/kg/day, including 1.6–1.7 g/kg of carbohydrates, 1.2–1.3 g/kg of protein, and 0.45–0.5 g/kg of fat during the 9-day period. The HMB group consumed 3 g/day HMB. Body composition measurement, isometric mid-thigh pull (IMTP), and a simulated boxing match were performed at baseline and on days 7, 8, and 9. Fasting blood samples were collected on the day before day 1 and on days 7, 8, and 9. Body mass was significantly decreased after the 6-day weight loss period (HMB group: baseline: 69.4 ± 11.2 kg, day 7: 67.1 ± 11.2 kg; PLA group: baseline: 68.6 ± 12.1 kg, day 7: 65.7 ± 11.5 kg, $P < 0.05$) while it was unchanged on the 3-day competition period in both the groups. Fat-free mass in the HMB group was maintained throughout the 9-day period (baseline: 56.7 ± 9.3 kg, day 7: 56.3 ± 8.7 kg, day 9: 55.8 ± 9.5 kg) whereas it significantly decreased on days 7 and 9 compared to the baseline in the PLA group (baseline: 55.2 ± 6.4 kg, day 7: 54.1 ± 6.6 kg, day 9: 54.0 ± 6.6 kg, $P < 0.05$). In the PLA group, the average and maximal heart rates in round 1 and the average heart rate in round 2 on days 8 and 9 were significantly lower than those at baseline, while these parameters were unchanged in the HMB group. The maximal force and the rate of force development in the IMTP remained unchanged among the different timepoints in both the groups. The blood biochemical parameters were similar at any timepoint between the PLA and HMB groups. HMB supplementation during acute weight loss may preserve fat-free mass and maintain heart rate response in subsequent simulated matches in well-trained boxers. In addition, HMB supplementation had a nonsignificant effect on glucose, fat, and protein metabolism during energy restriction.

Keywords: Body composition, boxing, isometric mid-thigh pull, simulated boxing match

INTRODUCTION

Acute weight reduction prior to competitions is a prevalent strategy observed in combat sports. Research suggests that a significant proportion of combat sports athletes, ranging from 60% to 90%, engage in this practice, with an average body mass reduction of around 5%.^[1,2] For instance, a study conducted among Australian amateur boxers found an average body mass reduction of 3.6% over a period of 12 days leading up to competitions.^[3] Similarly, another

investigation focusing on international-level amateur boxers revealed an average body mass reduction of 5.2% within a 7-day timeframe before competitions.^[4] Elite wrestlers who participated in the U-23 World Championships reported an average body mass reduction of 3.84 kg during a period of 7 days.^[5] Rapid weight loss in boxers can impair anaerobic performance as peak and average power was significantly

***Address for correspondence:** Dr. Chen-Kang Chang,
No. 16, Section 1, Shaun-Shih Road, Taichung 404, Taiwan.
E-mail: wspahn@seed.net.tw

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decreased in Wingate test.^[6] In addition to loss in body fat, lean tissues are usually lost during the energy restriction period, which can impair exercise performance.^[1,7] For example, losing an average of 5.1% body mass during 3 days through energy and fluid restriction resulted in a decrease in performance in 3-min intermittent intensity exercise and an increase in blood urea concentration in combat sports athletes.^[8]

Beta-hydroxy-beta-methylbutyrate (HMB), a leucine metabolite, has been shown to increase skeletal muscle mass and muscle strength in various pathological conditions involving muscle wasting or weakness, such as cancer cachexia^[9] and chronic obstructive pulmonary disease.^[10,11] The mechanisms responsible for the anticatabolic effects of HMB include the activation of mammalian target of rapamycin (mTOR), phosphatidylinositol 3-kinase/protein kinase B (Akt), and growth hormone signaling pathways, the increased proliferation of satellite cells and biosynthesis of cholesterol, and the inhibition of ubiquitin-proteasome-dependent proteolysis and nuclear factor- κ B activity.^[12-14] Despite its role in preventing proteolysis, HMB is rarely investigated in athletes under catabolic conditions, such as energy restriction or intensive training. A study involving soldiers who underwent strenuous military training suggested that HMB supplementation increased muscle volume by attenuating the increases in the inflammatory cytokines tumor necrosis factor- α and interferon- γ .^[15] However, physical performance after the supplementation was not reported.

Boxing is a weight-categorized sport with high demands for endurance, strength, and explosive power.^[16,17] Amateur boxers must compete across multiple days in a tournament with a weigh-in followed by one match each day. This schedule is different from other combat sports in which all matches were finished in 1 day, and only one weigh-in was required. The multiple weigh-ins are likely to prevent boxers from aggressive body mass recovery between weigh-ins and matches, which is common in other combat sports.^[18] Consequently, the maintenance of fat-free mass and physical performance during a multiday tournament after acute body mass loss is crucial for boxing success. In addition, the ability to deliver a high-impact punch is crucial for boxers, as it enhances their probability of success. It has been shown that greater maximal strength and power in lower extremity can lead to higher punch impact force in elite amateur boxers.^[17]

Therefore, this study investigated the effects of HMB supplementation on body composition and boxing-specific performance in catabolic conditions in well-trained collegiate male boxers consuming a hypocaloric diet. The study included one simulated boxing match on each of the 3 consecutive days after a 6-day energy restriction period to mimic a real competition schedule. In addition, maximal strength and rate of force development in lower extremity were measured using isometric mid-thigh pull (IMTP).

MATERIALS AND METHODS

Ethics

The experimental procedures were in accordance with the Helsinki Declaration of 1975, as revised in 2000. All participants provided written informed consent before participating in this study. The study protocol was approved by the Institutional Review Board of Antai-Tian-Sheng Memorial Hospital (19-032-A).

Study design

This study adopted a body weight-matched single-blind parallel design. Each pair of participants with a similar body mass was randomly assigned to the HMB or placebo (PLA) group. This study comprised a 6-day weight loss period (days 1–6), followed by a 3-day competition period (days 7–9) to represent typical amateur boxing tournaments [Figure 1]. The HMB and PLA groups consumed the same hypocaloric diet during the 9-day study period. The participants in the HMB group consumed 3 g/day calcium HMB from day 1 to day 9. Body composition and IMTP were measured in the morning on days 1, 7, 8, and 9. In the afternoon at the baseline and on days 7, 8, and 9, the participants underwent a simulated boxing match. The simulated match had similar movement patterns to international amateur boxing matches. The heart rates during the simulated matches and rate of force development in IMTP were used to evaluate physical performance.

Participants

Twelve male college boxers (age: 21.0 ± 1.8 years; height: 1.70 ± 0.06 m) from the National Taiwan University of Sport, Taichung, Taiwan, participated in this study. Each pair of participants with a similar body mass was randomly assigned to the HMB or PLA group. Each group contained six participants. At the baseline, the two groups had similar age (HMB: 21.3 ± 1.8 years vs. PLA: 20.7 ± 1.9 years), height (HMB: 1.70 ± 0.07 m vs. PLA: 1.71 ± 0.06 m), body mass (HMB: 69.4 ± 11.2 kg vs. PLA: 68.6 ± 12.1 kg), and body mass index (HMB: 23.7 ± 1.8 kg/m² vs. PLA: 23.4 ± 4.0 kg/m²). All participants had at least 5 years of experience in competitive boxing training and had competed nationally or internationally. All participants had experience in rapid body mass loss with energy restriction. The exclusion criteria included consuming protein or amino acid supplementation within the previous 3 months and those with cardiovascular, musculoskeletal, or other chronic diseases.

Dietary intervention and supplementation

The diet consumed during the 9-day study period contained 16 kcal/kg/day, including 1.6–1.7 g/kg of carbohydrates (41%–43% energy), 1.2–1.3 g/kg of protein (30%–32% energy), and 0.45–0.5 g/kg of fat (26%–28% energy) in both the groups. The food was purchased from a local diner and convenience stores. The participants came to the laboratory to finish all food at approximately 08:00, 12:00, and 18:00 each day. The participants were prohibited from consuming any food not provided by the research personnel. The participants

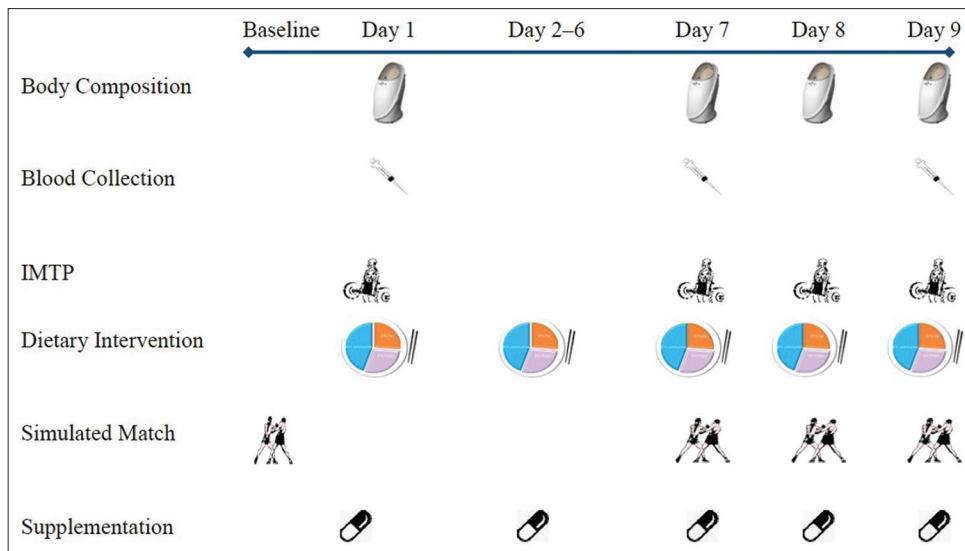


Figure 1: Study design. Days 1–6: weight loss period; Days 7–9: competition period (days 7–9). Blood collection: fasting blood samples collected in the morning. Dietary intervention: 16 kcal/kg/day, including 1.6–1.7 g/kg of carbohydrates, 1.2–1.3 g/kg of protein, and 0.45–0.5 g/kg of fat. Simulated match: each simulated match consisted of three 3-min rounds and each round consisted of 6 × 30-s sets of boxing-specific movements. Supplementation: β -hydroxy- β -methylbutyrate or placebo. IMTP: Isometric mid-thigh pull.

were instructed not to use dehydration to manipulate body mass and were allowed to drink water *ad libitum*. The participants in the HMB group consumed 1 g calcium HMB monohydrate in capsules (Optimum Nutrition, Downers Grove, IL, USA) after breakfast, lunch, and dinner at a daily dose of 3 g. The participants in the PLA group consumed the same number of capsules containing maltodextrin (Chung-Yu Biotechnology, Taichung, Taiwan).

Blood sample collection and analysis

On days 1, 7, 8, and 9, the participants came to the laboratory at 08:00 after an overnight fast. An aliquot of 10-mL fasting venous blood sample was collected in tubes containing ethylenediaminetetraacetic acid in the morning at the baseline and on days 7 and 9 [Figure 1]. After centrifugation at 1,500 g for 15 min at 4°C, the plasma samples were stored at –70°C until further analysis. Commercial kits were used to analyze plasma concentrations of glucose (Shino Co., Tokyo, Japan), triglyceride (Fujifilm Wako Pure Chemical Co., Osaka, Japan), glycerol (Randox Laboratories, Antrim, UK), non-esterified fatty acids (Randox Laboratories), β -hydroxybutyrate (Randox Laboratories), and urea (Randox Laboratories). The analyses were performed using an automatic analyzer (Hitachi 7020, Tokyo, Japan).

Body composition analysis

Body composition was analyzed in the morning on days 1, 7, 8, and 9 after the collection of blood samples [Figure 1]. Body mass, fat mass, and fat-free mass were measured using air displacement plethysmograph (Bod Pod, COSMED, Rome, Italy), according to the manufacturer's recommendations. During the measurement, the participants wore a swim cap and skin-tight compression shorts only and removed all body accessories.

Isometric mid-thigh pull

The participants underwent IMTP after measuring body composition at the baseline and on days 7, 8, and 9 [Figure 1]. The participants stood on a force platform (Kistler, Munich, Switzerland) above an immovable bar, which was set to a height where the hip angle was $155^\circ \pm 5^\circ$, and the knee angle was $125^\circ \pm 5^\circ$. The participants pulled the bar as hard and fast as possible and maintained a maximal effort for approximately 5 s while maintaining steady angles in the hip and knee joints.^[19] Three trials were performed in each test with a 2-min rest between trials. The maximal force and the rate of the development of vertical ground reaction force at 50, 100, and 200 ms were calculated. The trial with the highest maximal force was used for analysis. After finishing the IMTP, the participants consumed breakfast, followed by lunch at 12:00.

Simulated boxing matches

A simulated boxing match was undertaken in the afternoon at the baseline and on days 7, 8, and 9. The participants came to the laboratory at 13:30. Participants arrived at the laboratory at 13:30 and, upon arrival, each participant provided a prematch urine sample. All urine samples showed gravity below 1.015 as shown in urine test strips (AUTION Sticks 10EA, Arkray, Minneapolis, MN, USA), indicating that all participants were euhydrated before each simulated match.^[20]

The simulated match was according to a previous study with slight modifications.^[21] The internal load and performance-based efforts in this protocol have been validated.^[22] A decagon was marked with a target in the center on the floor of a boxing ring [Figure 2]. Each simulated match consisted of three 3-min rounds with 1-min breaks between rounds. Each round consisted of 6 × 30-s sets of boxing-specific movements. Each set consisted of 10 attack and 6 defense activities [Table 1].

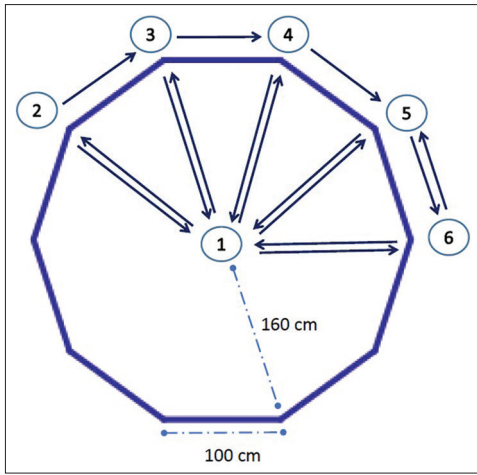


Figure 2: Schematic of the simulated boxing match. The numbers represented the starting and end position while the arrows showed the movement direction of each activity in Table 1.

Table 1: The 30-s set of movements in the simulated boxing matches

Starting position	Movement direction	End position	Attack activity	Defense activity
2	Left	3		
3	Forward	1	One-two punch	
1	Backward	3		Feet
3	Left	4		
4	Forward	1	Jab	
1	Backward	4		Hands
4	Left	5		
5	Forward	1	Rear cross	
1	Backward	5		Trunk
5	Left	6		
6	Forward	1	Rear cross, lead hook, rear cross	
1	Backward	6		Feet
6	Right	5		
5	Forward	1	One-two uppercut	
1	Backward	5		Hands
5	Left	6		
6	Forward	1	Jab	
1	Backward	6		Trunk

Each 3-min round is consisted of six sets while each match is consisted of three 3-min rounds

The starting and end position and movement direction of each activity are shown in Figure 2. One co-author (S. Y. Kao) stood at the center of the decagon [position 1 in Figure 2] while wearing a punch pad in each hand. The participants moved forward from position 2 to attack the target, then moved backward to position 2 with defensive movements, and then moved laterally to the next corner (position 3). At the end of the first set, the participants reached position 6, from which the next set of activities would be repeated. The vocal instruction of the movements was recorded in an MP3 file. The participants were instructed to follow the instructions with maximal attack

and defense efforts. The heart rates during the simulated matches were recorded using a heart rate monitor (H10, Polar Electro, Kempele, Finland) to calculate the maximal and average heart rates of each round.

Statistical analysis

Data are presented as means ± standard deviations. Nonparametric methods were used for analysis because the data were not normally distributed according to the Shapiro–Wilk test. The differences among timepoints within the same group were analyzed using the Friedman test. If a significant time effect was found, the Wilcoxon signed-rank test was used for *post hoc* comparison. The percent changes in body composition and blood parameters on days 7, 8, and 9 from the baseline were calculated. The differences in the percent changes between the HMB and PLA groups were compared using the Mann–Whitney *U*-test. The differences in the maximal and average heart rates in the simulated matches at the same timepoints between the HMB and PLA groups were also analyzed using the Mann–Whitney *U*-test. Statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, New York, USA). *P* < 0.05 was used to denote statistical significance.

RESULTS

This study comprised a 6-day body mass loss period (days 1–6), followed by a 3-day competition period (days 7–9). The effects of HMB supplementation on body composition, heart rate response in simulated matches, maximal strength and rate of force development in lower limbs, and blood biochemical parameters were investigated. The body composition at the baseline and on days 7, 8, and 9 in the HMB and PLA groups is shown in Table 2. In both the groups, body mass was significantly decreased after the 6-day weight loss period (HMB group: *P* = 0.028; PLA group: *P* = 0.027), while it was maintained on the following 3-day competition period. The magnitude of body mass loss on days 7, 8, and 9 in the HMB group showed a significant time effect. However, the *post hoc* analysis did not show any significant difference between timepoints. The magnitude of body mass loss compared to the baseline was similar between the two groups on days 7, 8, and 9.

Fat-free mass in the HMB group was maintained throughout the 9-day period, whereas it was significantly decreased on days 7 (*P* = 0.046) and 9 (*P* = 0.028) compared with that at the baseline in the PLA group [Table 2]. The magnitude of fat-free mass loss compared to the baseline was similar between the two groups on days 7, 8, and 9. Fat mass and body fat percentage showed an insignificant decreasing trend in both the groups. The magnitude of loss in fat-free mass, fat mass, and body fat percentage on days 7, 8, and 9 compared with that at the baseline was not significantly different between the two groups.

The average and maximal heart rates in each round of the simulated boxing matches at the baseline and on days 7, 8, and 9 are presented in Table 3. The average and maximal heart

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Table 2: Body composition at the baseline and on days 7, 8, and 9 in the HMB and PLA groups

	Group	Baseline	Day 7	Day 8	Day 9	P ^b
Body mass (kg)	HMB	69.4±11.2	67.1±11.2*	66.9±11.2*	66.8±11.1*	0.002
	PLA	68.6±12.1	65.7±11.5*	66.0±11.1*	66.3±10.6*	0.008
Change ^c (%)	HMB	-	-3.30±0.91	-3.67±1.14	-3.74±1.44	0.032
	PLA	-	-4.18±0.92	-3.73±1.81	-4.01±2.01	0.438
Fat free mass (kg)	HMB	56.7±9.3	56.3±8.7	56.0±9.0	55.8±9.5	0.384
	PLA	55.2±6.4	54.1±6.6*	54.1±6.0	54.0±6.6*	0.029
Change ^c (%)	HMB	-	-0.57±1.69	-1.10±1.43	-1.65±1.24	0.664
	PLA	-	-2.03±2.01	-0.95±1.57	-2.28±1.53	0.223
Fat mass (kg)	HMB	12.6±4.8	10.8±4.0	10.8±3.9	11.0±3.9	0.169
	PLA	13.3±6.3	11.6±5.8	11.3±5.9	11.6±5.8	0.091
Change ^c (%)	HMB	-	-13.66±11.40	-13.45±13.56	-12.20±11.39	0.878
	PLA	-	-13.43±12.28	-16.64±11.39	-12.74±12.56	0.260
Body fat (%)	HMB	18.1±5.5	15.9±4.5	16.0±4.4	16.4±4.9	0.172
	PLA	18.9±5.1	17.0±5.4	16.5±5.6	17.4±5.3	0.100
Change ^c (%)	HMB	-	-10.80±10.36	-9.99±12.03	-8.77±9.28	0.846
	PLA	-	-9.87±12.09	-13.47±10.91	-7.86±10.78	0.321

*P < 0.05 significantly different from the baseline in the same trial using the Wilcoxon signed-rank test. ^bFriedman test, ^cChange from the baseline value. All data are expressed as mean ± standard deviation. HMB: β-hydroxy-β-methylbutyrate, PLA: Placebo

Table 3: Maximal and average heart rates in rounds 1, 2, and 3 in the simulated boxing matches at the baseline and on days 7, 8, and 9 in the HMB and PLA groups

	Group	Baseline	Day 7	Day 8	Day 9	P ^b
R1 HR _{avg} (bpm)	HMB	142.8±18.6	139.5±20.5	130.3±16.0	129.6±17.2	0.229
	PLA	152.6±13.1	133.6±14.9	132.7±13.2*	124.5±15.6*	0.046
R1 HR _{max} (bpm)	HMB	155.8±17.5	144.5±10.6	140.1±17.2	142.8±25.1	0.172
	PLA	172.0±15.1	158.8±13.3	151.0±17.1*	141.0±21.2*	0.001
R2 HR _{avg} (bpm)	HMB	158.3±23.0	150.0±13.8	148.3±14.2	144.5±18.9	0.308
	PLA	164.0±15.6	158.2±9.6	151.8±15.7*	137.3±17.5*	0.008
R2 HR _{max} (bpm)	HMB	169.3±19.3	157.2±16.2	156.3±14.5	155.0±21.9	0.086
	PLA	175.3±14.5	170.0±10.1	164.0±19.2	153.3±18.6	0.102
R3 HR _{avg} (bpm)	HMB	161.8±21.6	154.0±10.0	153.3±13.9	150.7±19.1	0.457
	PLA	175.0±12.0	158.7±14.3	156.8±13.7	152.2±17.9	0.134
R3 HR _{max} (bpm)	HMB	171.0±19.6	162.5±10.8	161.7±14.6	161.5±23.6	0.457
	PLA	186.7±10.7	171.0±16.5	170.2±14.1	164.3±18.7	0.119

*P < 0.05 significantly different from the baseline in the same trial using the Wilcoxon signed-rank test. ^bFriedman test. All data are expressed as mean ± standard deviation. HMB: β-hydroxy-β-methylbutyrate, PLA: Placebo, R1: Round 1, R2: Round 2, R3: Round 3, HR_{max}: Maximum heart rate, HR_{avg}: Average heart rate

rates in round 1 and the average heart rate in round 2 in the PLA group on days 8 and 9 were significantly lower than those at baseline, whereas these parameters were unchanged in the HMB group. However, the average and maximal heart rates were not significantly different between the two groups in any round. The maximal force and the rate of force development at 50, 100, and 200 ms in the IMTP remained unchanged among the different timepoints in the HMB and PLA groups [Table 4].

The blood biochemical parameters at the baseline and on days 7 and 9 in the HMB and PLA groups are shown in Table 5. Plasma triglyceride concentrations were significantly decreased on days 7 and 9 compared with those at the baseline in both the groups. Plasma β-hydroxybutyrate concentrations were significantly elevated on days 7 and 9 in the PLA group, whereas they only showed an insignificant trend of increase

in the HMB group. However, the percent changes in all blood parameters from the baseline to days 7 and 9 were similar between the two groups.

DISCUSSION

Most boxers do not practice gaining significant body mass after a weigh-in because they must meet the weight category again the next day. This study adopted a similar dietary intervention to mimic the international competitions as no aggressive recovery was provided before the simulated matches. The results of this study showed that HMB supplementation during a 9-day acute body mass loss and competition period may preserve fat-free mass in well-trained collegiate boxers consuming a hypocaloric diet. In addition, HMB supplementation may help maintain the maximal and average heart rates during simulated boxing

Table 4: Maximal force and rate of force development in the isometric mid-thigh pull at the baseline and on days 7, 8, and 9 in the HMB and PLA groups

	Group	Baseline	Day 7	Day 8	Day 9	P ^b
Maximal force (N)	HMB	2,237±627	2,264±522	2,159±490	2,163±452	0.172
	PLA	2,254±506	2,265±436	2,354±413	2,022±251	0.072
RFD 50 ms (N/s)	HMB	14,793±3,440	12,617±2,251	13,916±2,379	13,327±2,443	0.572
	PLA	14,904±5,571	12,208±3,159	14,204±3,463	13,963±5,554	0.284
RFD 100 ms (N/s)	HMB	9,923±2,926	9,012±2,998	10,944±3,499	10,361±3,342	0.457
	PLA	9,162±2,765	8,418±2,052	10,278±2,049	10,080±2,308	0.066
RFD 200 ms (N/s)	HMB	7,789±2,741	6,401±2,102	7,260±1,693	6,750±1,109	0.392
	PLA	6,773±2,273	5,895±1,831	7,198±2,103	6,537±1,331	0.457

**P* < 0.05 significantly different from the baseline in the same trial using the Wilcoxon signed-rank test. ^bFriedman test. All data are expressed as mean ± standard deviation. HMB: β-hydroxy-β-methylbutyrate, PLA: Placebo, RFD: Rate of force development

Table 5: Blood biochemical parameters at the baseline and on days 7 and 9 in the HMB and PLA groups

	Group	Baseline	Day 7	Day 9	P ^b
Glucose (mmol/L)	HMB	5.29±0.45	4.79±0.31	4.72±0.34	0.331
	PLA	5.63±0.60	4.77±0.63	4.98±0.77	0.115
Triglyceride (mmol/L)	HMB	0.66±0.20	0.48±0.15*	0.38±0.07*	0.006
	PLA	0.86±0.52	0.61±0.54*	0.31±0.13*	0.016
Nonesterified fatty acids (mmol/L)	HMB	0.46±0.21	0.63±0.22	0.73±0.18	0.115
	PLA	0.37±0.23	0.65±0.24	1.03±0.64	0.135
Glycerol (mmol/L)	HMB	48.83±18.03	51.76±21.80	77.20±34.34	0.311
	PLA	44.67±19.34	51.27±21.80	110.71±79.77	0.846
β-hydroxybutyrate (mmol/L)	HMB	0.08±0.07	0.27±0.24	0.34±0.18	0.069
	PLA	0.05±0.02	0.31±0.18*	0.53±0.42*	0.042
Blood urea nitrogen (mmol/L)	HMB	6.55±2.38	6.82±1.45	7.50±2.51	0.513
	PLA	6.78±2.77	6.76±1.17	6.95±1.12	0.846

**P* < 0.05 significantly different from the baseline in the same trial using the Wilcoxon signed-rank test. ^bFriedman test. All data are expressed as mean ± standard deviation. HMB: β-hydroxy-β-methylbutyrate, PLA: Placebo

matches after acute body mass loss. HMB supplementation had nonsignificant effect on maximal force and rate of force development and glucose, fat, and protein metabolism.

HMB has been shown to preserve muscle mass and function in patients with clinical conditions characterized by loss of skeletal muscle mass, such as cancer and chronic obstructive pulmonary disease.^[9,10] The results of this study revealed that HMB supplementation may also prevent the loss in fat-free mass and maintain the heart rate response during the short-term energy restriction. To the best of our knowledge, only one study has investigated the effects of HMB supplementation on body composition and physical performance during acute weight loss in athletes. HMB supplementation during a 3-day energy restriction period may help reduce body fat but had no effect on fat-free mass, grip strength, or anaerobic performance in female judo athletes.^[23] The different responses to HMB in body composition and exercise performance may result from sex differences or the duration of the supplementation. In untrained men, HMB with protein intake can also improve net protein balance after a 36-h fast.^[24] Animal and cell studies also supported the anticatabolic effect of HMB. An animal study reported that HMB supplementation during a 6-week energy restriction period prevented the decreases in the cross-sectional area in gastrocnemius and grip strength.^[25] Furthermore, HMB prevented protein degradation by activating

the Akt and mTOR pathways in C2C12 myotubes incubated with a serum-free medium to mimic starvation.^[26]

The effects of HMB on body composition while consuming adequate energy were mixed. In combat sports athletes, HMB supplementation during a 12-week training period with adequate energy intake significantly increased lean body mass and decreased fat mass while exhibiting larger improvements in endurance and anaerobic performance.^[27] The Position Stand from the International Society of Sports Nutrition stated that HMB can enhance recovery by attenuating exercise-induced skeletal muscle damage in trained and untrained populations.^[28] However, the anabolic effect of HMB may be more significant in individuals who have just begun resistance training.^[29] In contrast, a recent systematic review and meta-analysis revealed that HMB supplementation during several weeks of training did not significantly affect body composition or muscular strength in athletes consuming adequate energy.^[30]

The daily energy intake (approximately 1100 kcal) in this study was similar to the reported values (approximately 1000 kcal/day) in combat sports athletes during the acute weight loss period.^[31,32] The low energy intake limited dietary protein content to 1.2–1.3 g/kg/day, which was lower than the recommended levels (1.6–2.4 g/kg/day) to prevent the

loss in fat-free mass during energy restriction in athletes or resistance-trained individuals.^[33] The energy restriction and low protein intake resulted in a significant decrease in fat-free mass in the PLA group. On the other hand, the HMB supplementation was able to prevent the loss in fat-free mass with the same energy and protein intake.

Although the number of attack and defense movements was the same in each simulated boxing match, the participants in the PLA group showed significantly lower heart rate responses in rounds 1 and 2 after acute body mass loss on days 8 and 9 than the baseline values, whereas the heart rate responses remained unchanged in the HMB group. The heart rate responses to the same level of ratings of perceived exertion can serve as indicators of fatigue. Lower heart rates at the same level of ratings of perceived exertion were found during a 3-week cycling grand tour^[34] and sleep restriction.^[35] In addition, increased ratings of perceived exertion to heart rate ratio were observed during a 7-day training camp when the training load was increasing, which also indicated a decreased heart rate response during fatigue. A rest day during the camp was able to decrease the ratings of perceived exertion to heart rate ratio.^[36] The participants in both the groups were instructed to apply their greatest effort during the simulated matches, which should result in similar ratings of perceived exertion. However, the participants in the PLA group were unable to reach the heart rate levels in rounds 1 and 2 on the last 2 days, whereas those in the HMB group maintained the heart rate responses. These results indicated that HMB supplementation may help maintain heart rate response during energy restriction in collegiate boxers.

The simulated matches in this study were designed to mimic the intensity of international boxing competitions. Our simulated matches included 60 punches and 36 defensive movements per round, which were similar to 61.0–70.8 total punches and 7.1–9.2 total defense movements per round in international matches.^[37] In addition, 63 punches and 10.8 defensive movements per round in the 2015 World Championship matches were reported.^[38] International boxing matches elicit high heart rate responses and high demands for anaerobic and aerobic energy systems. In elite boxers, the average heart rate during a match corresponded to 93% of the maximal heart rate, and the average blood lactate concentration was 8.87 mM after the match.^[39] Another study revealed that the average oxygen consumption was 89% $\text{VO}_{2\text{max}}$ during boxing matches.^[40] A simulated boxing match similar to our protocol elicited an average heart rate of 165 ± 20 bpm,^[21] which was similar to our results at the baseline. The maximal and average heart rates in the simulated matches in this study were lower than the previously reported values in international matches, particularly after acute body mass loss on days 8 and 9. The lower heart rate response was possibly due to the lack of body contact and competition stress and lower glycogen contents after the energy restriction period.

The IMTP is a common and reliable method for quantifying the maximal strength and rate of force development in athletes.^[41]

The neuromuscular characteristics obtained in the IMTP are significantly correlated with various athletic performances, such as jumping, throwing, and change of direction.^[42] This study showed that acute body mass loss had no significant effect on the maximal force and rate of force development in the lower limbs of boxers. Despite the preservation of fat-free mass, HMB supplementation did not affect the results of the IMTP. To the best of our knowledge, no study has investigated the effects of HMB on muscular performance during energy restriction in humans. An animal study suggested that HMB supplementation during a 6-week energy restriction period prevented the decreases in grip strength.^[25] While consuming adequate energy, a 12-week HMB supplementation may increase the peak and average power in the Wingate test in combat sports athletes.^[27] Conversely, a meta-analysis revealed that HMB supplementation during training had a nonsignificant effect on muscular strength in athletes consuming adequate energy.^[30]

The plasma concentrations of glycerol, non-esterified fatty acid, and 3-hydroxybutyrate showed a trend of increase during energy restriction, indicating slightly elevated lipolysis. The participants in both trials remained euglycemic during the 9-day study period. The blood biochemical parameters were similar at any timepoint between the PLA and HMB groups, indicating that HMB supplementation had a nonsignificant effect on glucose, fat, and protein metabolism during energy restriction. In agreement with our results, Crowe *et al.* showed that supplementation of 3 g/d HMB with adequate energy intake for 6 weeks had no significant effect on plasma concentrations of triglyceride and urea in highly trained athletes.^[43]

This study has several limitations. First, the sample size is small due to the difficulty in recruiting participants during the COVID-19 pandemic. The second wave of the proposed cross-over design was canceled due to the same reason. Second, the simulated matches may not fully represent the physical demands in real boxing competitions. Third, fluid intake was uncontrolled, which can be another factor that influences body mass. However, the participants were all euhydrated, according to urine gravity, before the simulated matches to prevent the potential negative effects of dehydration on boxing performance.^[44] Fourth, an additional recovery meal was not provided after weigh-in, which is different from the common dietary interventions on match days.

CONCLUSION

HMB supplementation may preserve fat-free mass during acute energy restriction and maintain heart rate response in subsequent simulated matches in well-trained boxers. Future studies can investigate the performance of boxers in real boxing matches after HMB supplementation using video analysis.

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Conflicts of interest

There are no conflicts of interest.

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