

# Effect of HMB-FA Supplementation on Physiological Recovery Markers in Elite Wrestlers: A Double-Blind Placebo-Controlled Study

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## Abstract

β-Hydroxy-β-Methylbutyrate Free Acid (HMB-FA) has been reported to improve skeletal muscle recovery after high-intensity exercise and attenuate blood markers of skeletal muscle damage. Therefore, this study was conducted to determine the effect of HMB-FA supplementation on physiological recovery markers in elite wrestlers. Twenty elite healthy wrestlers (aged 19–26 years) were randomly assigned to Exercise+HMB-FA (EXE+S, n=10) and Exercise+Placebo (EXE+P, n=10) groups for a simulated wrestling protocol. Compared to the EXE+P group, the EXE+S group showed significant decreases in physiological recovery markers such as Creatinine (Cr), 3-Methylhistidine (3-MH), urinary 3-Methylhistidine/Creatinine ratio (3-MH/CR), Lactate Dehydrogenase (LDH), and Creatine Kinase (CK), with noticeable reductions ( $p<0.05$ ). Additionally, there were significant reductions in cortisol, and significant enhancements in Insulin-like Growth Factor-1 (IGF-1) and IGF-1/cortisol ratio in the EXE+S group ( $p<0.05$ ). Finally, the Perceived Recovery Status (PRS) as another physiological recovery marker in the EXE+P group was significantly reduced compared to the EXE+S group ( $p<0.05$ ), indicating a negative impact on recovery without HMB-FA supplementation. HMB-FA may attenuate muscle damage and improve recovery by modifying the cortisol, IGF-1, and IGF-1/cortisol ratio and increase PRS in the simulated wrestling protocol.

**Keywords:** Wrestler • Supplement • Recovery • HMB-FA • Polydextrose

## Introduction

Wrestling, a time honored combat sport that dates back to 708 BC, requires exceptional strength, power, and balance as athletes execute a series of sport specific whole body movements during a 7 minute match with limited interruptions. Furthermore, the anaerobic system maintains short rapid bursts of maximal power activities during the match, while the aerobic system manages the wrestler's ability to maintain effort throughout the contest and accelerates the recovery process between successive matches. Due to the high intensity nature of wrestling and the limited recovery time between rounds and matches that can exacerbate physiological damage and reduce performance, wrestlers and their coaches must investigate recovery strategies and factors that can improve performance. Achieving an ideal balance between training, competition, and physiological recovery is crucial to optimize performance in wrestling [1].

Legal ergogenic aids, which possess properties that increase performance and/or accelerate recovery between matches, can benefit wrestler recovery profiles. HMB-FA (beta-hydroxy-beta-methylbutyrate free acid) is a dietary supplement known for its anabolic effects on protein metabolism. HMB-FA has demonstrated promising effects on exercise performance, improvements in performance recovery, muscle recovery, and protein synthesis in resistance trained individuals, professional athletes, and combat sports athletes. However, no previous studies have specifically examined the acute effects of HMB-FA supplementation on physiological recovery markers in wrestlers following a specific wrestling protocol [2].

In this study, our objective is to investigate the impact of short-term HMB-FA supplementation on the physiological recovery markers in elite wrestlers. We hypothesize that HMB-FA supplementation will improve physiological recovery markers such as cortisol, IGF-1, IGF-1/cortisol ratios, and PRS, and will decrease other physiological recovery markers such as Cr, 3-MH, 3-MH/Cr, LDH, and CK in elite wrestlers. Our findings will contribute to the current body of literature and provide practical implications for wrestlers and coaches seeking to improve recovery and performance [3].

## Materials and Methods

### The experimental approach to the problem

This study was a randomized, double-blind, placebo-controlled, and diet-controlled experiment to study the effects of HMB-FA supplementation on physiological recovery markers in elite wrestlers. The UMWAPT wrestling specific protocol includes a warm-up phase and a main activity step (the main activity stage had divided into four stages and three continuous parts). Each wrestler repeated the main activity step five times. The rest time between each main activity was 30 minutes. Serum Creatine Kinase (CK), Lactate Dehydrogenase (LDH), Insulin-like Growth Factor-1 (IGF-1), cortisol, and serum IGF-1/cortisol ratio values were measured from blood samples collected at various time points. These time points included before supplement intake, after supplement intake, and immediately following the first, third, and fifth repetitions of the simulated wrestling protocol. Urine samples were collected to measure urine Creatinine (Cr), 3-Methylhistidine (3-MH), and 3-Methylhistidine/Creatinine ratio (3-MH/Cr) before supplementation, after supplementation, and immediately after the first, second, third, fourth, and fifth repetitions of the simulated wrestling protocol. Finally, the Perceived Recovery Status (PRS) was evaluated immediately before the first, second, third, fourth, and fifth repetitions of the specific wrestling protocol [4].

### Subjects

Among the trained volunteer wrestlers eligible to participate in the study, with an  $\alpha=0.05$ , an effect size=0.90, and a power of 80%, a sample size of 20 was recommended. Therefore, 20 wrestlers aged 19-26 years with an average of the country's adult league and international competition experience of  $4.0 \pm 2.0$  years were randomly divided into two groups: Exercise supplement (EXE + S, n=10) and exercise placebo (EXE + P, n=10) (Figure 1). To be eligible, they had to be in the last of the 3 weeks of abstinence from wrestling on the basis of the annual periodization of training (off-season phase). The wrestlers six months before enrolling in the study had not consumed alcohol, drugs, cigarette, caffeine, carnitine, or supplements. They also avoided eating protein-rich foods for 72 hours before collecting urine and blood samples. In

this study, participants were also excluded from the study if they used anabolic steroids or hormonal precursors in the last 6 months. There were no differences between the participants for age; height; weight;  $VO_{2max}$ ; resting heart rate; body fat; body mass index; or recovery step 1 at the beginning of the study (Table 1). Written informed consent was obtained from each wrestler prior to the investigation and the wrestlers were informed of the benefits and risks of the investigation prior to signing the institutionally approved informed consent document to participate in the study. The research protocol was approved by the medical ethics committee of Allameh Tabatabaie University (IR.ATU.REC.1398.010). All protocols were performed in accordance with the relevant guidelines and regulations of the captioned ethics committee [5].

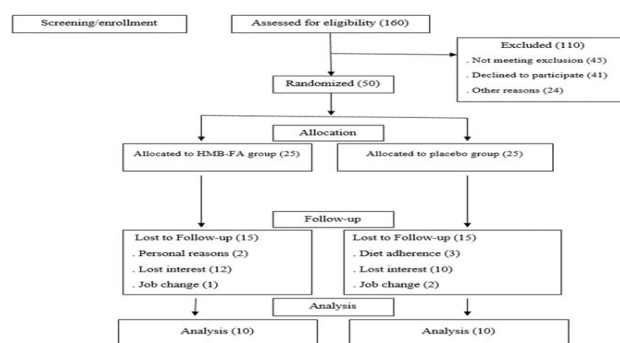


Figure 1. Follow-up diagram.

**Table 1.** Comparison of the mean changes of demographic variables in the EXE+S and EXE+P groups. BMI: Body mass index,  $VO_{2max}$ : Maximum oxygen consumption, bpm: Heart rate per minute, SD: Standard Deviation.

Variables	EXE+S (n=10) Mean $\pm$ SD	EXE+P (n=10) Mean $\pm$ SD	P-value
Age (years)	24.1 $\pm$ 2.84	23.7 $\pm$ 2.67	0.75
Height (cm)	168 $\pm$ 6	174 $\pm$ 2.2	0.67
Weight (kg)	76.9 $\pm$ 12.48	78.5 $\pm$ 9.58	0.56
Body fat (%)	12.88 $\pm$ 1.28	12.86 $\pm$ 2.05	0.97
$VO_{2max}$ (ml $kg^{-1}min^{-1}$ )	60.24 $\pm$ 5.13	63.28 $\pm$ 4.78	0.78
Resting heart rate (bpm)	48.5 $\pm$ 3.93	47.6 $\pm$ 3.65	0.45
BMI (kg/m <sup>2</sup> )	25.27 $\pm$ 4.17	25.78 $\pm$ 4.38	0.79
Recovery step 1	6.1 $\pm$ 1.72	4.4 $\pm$ 0.51	0.1

## Procedures

### Anthropometrics, and maximal oxygen consumption ( $VO_{2max}$ )

Height and body weight were measured to the nearest 0.1 cm and the nearest 0.1 kg, respectively, using a fixed Sanny brand stadiometer and a Seca digital scale (SECA 222; SECA, Hamburg, Germany). Body mass index and percentage of body fat were measured using body composition analyzer (InBody-570, South Korea). Heart rate was monitored telemetrically throughout all exercise protocols using an HR monitor ((Polar H9/H10, Finland) (AXN500). All wrestlers underwent incremental running tests (h/p cosmos pulsar treadmill, sports and medical GmbH, Nussdorf-Traunstein, Germany) to determine  $VO_{2max}$  [6].

### $VO_{2max}$

All wrestlers underwent incremental running tests to determine  $VO_{2max}$ . The initial speed was set at 4 kmh<sup>-1</sup> and after 3 min increased to 8 kmh<sup>-1</sup>. After that point, the speed of the treadmill increased progressively by 2 kmh<sup>-1</sup> every 3 min until voluntary exhaustion. Two of the following four criteria were required for a test to be considered maximal: A plateau in  $VO_2$  despite increasing workload; Respiratory Exchange Ratio (RER)  $\geq$  1.10; maximal heart rate within 10 beats of the maximum predicted by age (max HR=(208 - (0.7  $\times$  age in the year))); and RPE 17 [7].

### HMB-FA supplementation

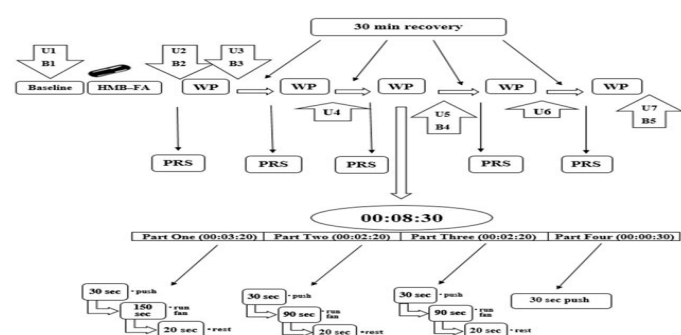
The HMB-FA supplement (marketed as BetaTor®; metabolic technologies) contained Litesse® polydextrose,  $\beta$ -hydroxy  $\beta$ -methylbutyrate FA, reverse osmosis water, debittering agent, orange flavor, stevia extract, potassium carbonate, and potassium sorbate. Each capsule contained 1 g of  $\beta$ -hydroxy- $\beta$ -methylbutyrate in the free acid form.

The EXE+S group received HMB-FA in three capsules three times day and night (24:00, 06:00 am, and 30 minutes before the Wrestling Specific Protocol) (total HMB-FA dose of 3 g). For the EXE+P group, each serving of placebo contained one gram of polydextrose and was identical in appearance and taste to the HMB-FA supplement. Both the supplement and placebo were administered double-blind. Both supplements and placebo were in capsule form and were matched for taste, appearance, and calories. An outside member naive to the nature of this study prepackaged each serving of the assigned supplement in coded bottles, and this person who was not related to the research team was assigned to deliver the supplements to subjects to ensure a double-blind design. The coding information was sealed in an envelope and opened once data collection was complete [8].

### Diet control

To determine the possible impact of the diet on the study results and any changes in the diet throughout the study, the nutrient intake of the wrestlers was evaluated using a validated 24-hour recall food frequency questionnaire. Wrestlers were required to maintain their normal diet during the study period and instructed to consume a diet as similar as possible on each sampling day. Participants were required to avoid any prescription or over-the-counter medications/supplements and foods that could affect their physical function 72 hours before and during the study. The diet intake data were analyzed using diet analysis software (Nutrition tracker Pro\_v2.0.2\_Apkpure) to determine the total energy intake, protein, carbohydrate, and fat intake of the subjects. Information on the use of medications/supplements was also obtained through a self-reported questionnaire [9].

**Wrestling-specific protocol:** The wrestling specific protocol was held between 1:00 and 4:00 pm in the presence of wrestlers, coaches, and a group of wrestlers as spectators. Eligible subjects performed the wrestling specific protocol UMWAPT as follows. 1) To warm up, subjects were instructed to run in place for 2 minutes and 55 seconds. When the subjects reached that mark, they were to sprint in place for 5 seconds. After 5 seconds, they continued to jog in place for another 2 minutes and 55 seconds. There are three rounds of this warm-up. Afterward, the wrestler had an inactive recovery of one minute and 50 seconds. 2) The main activity step; a special training sledge was used to perform part of this step. A blocking sled is a piece of training equipment that is used to help wrestlers with their blocking techniques and to develop their strength. The sled is usually made out of metal, is heavily padded, and sits on skids (similar to a sled) so that it is the size of a wrestler's height and can be pushed. At that time, the blocking sled (Gilman one-man tackleback) was loaded with weights to a total weight (sled's actual weight added weights) equivalent to 2.5 times the wrestler's body weight. In this way, wrestlers who interact with the sled can practice the exercises of penetration, leverage drills, blocking, and other skills as if they were practicing against a real person. The wrestlers then stood up right near the sled and took their "holding point" on the sled. To ensure that the test mimicked the real wrestling movement as much as possible, each subject was allowed to use his own technique relevant to the position. The wrestlers pushed the blocking sled as far as they could for 30 seconds. Immediately after the wrestlers finished the initial 30-second sled push, they drilled double legs with a teammate for 2½ minutes (150 seconds) at a drill pace (roughly 20–25 double legs in total). The wrestlers then rested for 20 seconds before pushing the sled for a second time for 30 seconds as fast and far as possible. The wrestlers then immediately engaged in drilling half-nelsons with a pre-breakdown with a teammate for 1½ minutes (90 seconds) at a drill pace (roughly 15–20 repetitions). The wrestlers rested for 20 seconds and pushed the sled for a third time for 30 seconds as fast and far as possible. The wrestlers then immediately engaged in drill escapes with a teammate for 1½ minutes (90 seconds) at a drill pace (roughly 20–25 repetitions). Immediately after 20 seconds of rest, the wrestlers pushed the sled for the fourth and final time for 30 seconds as fast and far as possible. The wrestlers then supine for 15 minutes. Each wrestler repeated the main activity step five times. The rest time between each main activity was 30 minutes and after each main activity, the wrestlers rested in a supine position for 15 minutes (Figure 2) [10].



## Results

### Recruitment to trial

A total of 160 healthy trained volunteer wrestlers were screened for eligibility for the current trial. Of these, 110 subjects (68.8%) were excluded because they did not meet the inclusion criteria and the reasons for exclusion are shown in Figure 1. In addition, 41 wrestlers were eligible for admission to the trial but refused to participate. Fifty (31%) wrestlers were finally included, and of these 30 wrestlers (40%) were excluded. The most common reason for exclusion was the loss of interest [15].

### Dietary and medication intake

The dietary intakes of the wrestlers were similar in the two groups, and the dietary intakes between the groups or within the groups did not change over the study ( $P>0.05$ ). The wrestlers in the supplement group consumed a total of 3120 kcal per day, which consisted of 468 grams of carbohydrates, 69.33 grams of fat, and 156 grams of protein. In the placebo group, the wrestlers consumed a total of 3115 kcal per day, with their diet consisting of 467.25 grams of carbohydrates, 69.22 grams of fat, and 155.75 grams of protein. It's worth highlighting that the contribution of each of these sources was deemed to be 60% for carbohydrates, 20% for fats, and 20% for proteins across both groups [16].

### Physiological recovery markers

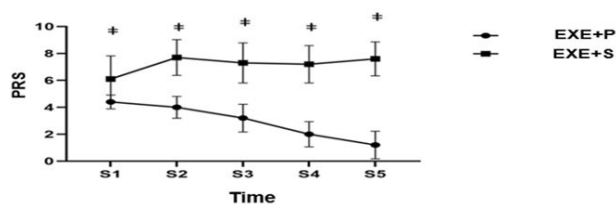
HMB-FA supplementation showed significant changes in physiological recovery markers (cortisol, IGF-1, IGF-1/Cort, PRS, Cr, 3-MH, 3-MH/Cr,

LDH and CK) in elite wrestlers ( $P<0.05$ ) (Tables 2 and 3). Serum levels of IGF-1 after supplementation (EXE+S=(+23.4%), EXE+P=(-6.77);  $p=0.001$ ), and immediately after the first (EXE+S=(+16.3%), EXE+P=(-6.49%);  $p=0.004$ ), the third (EXE+S= +1.44%), EXE+P=(-7.23%);  $p=0.04$ ) and the fifth (EXE+S=(+1.58%), EXE+P=(-14.89%);  $p=0.013$ ) repetitions of the simulated wrestling protocol in the EXE+S group were higher than in the EXE+P group (Table 2). Serum levels of IGF-1/Cort after supplementation (EXE+S=(+60.12%), EXE+P=(-16.07%);  $p=0.001$ ) and after the first (EXE+S=(+10.6%), EXE+P=(-55.58%);  $p=0.001$ ), the third (EXE+S=(-17.79%), EXE+P=(-71.65%);  $p=0.003$ ) and the fifth (EXE+S=(-29.03%), EXE+P=(-77.9%);  $p=0.001$ ) repetitions of the simulated wrestling protocol in the EXE+S group was higher than the EXE+P group; but the serum cortisol values in the EXE+S group compared to the EXE+P group after supplementation (EXE+S=(-21.6%), EXE+P=(+19.32%);  $p=0.006$ ) and after the first (EXE+S=(+13.22%), EXE+P=(+120%);  $p=0.002$ ), the third (EXE+S=(+52.27%), EXE+P=(+291%);  $p=0.008$ ) and fifth (EXE+S=(+60.33%), EXE+P=(+324%);  $p=0.002$ ) repetitions of simulated wrestling protocol was lower (Table 2). In the EXE+S group, the PRS score increased significantly in the early stages of the wrestling specific protocol ( $P<0.05$ ). As the steps continued, the PRS score showed a smaller decrease compared to the EXE+P group. In the EXE+P group compared to the EXE+S group, the PRS score decreased significantly ( $P<0.05$ ) (Figure 3) [17].

**Table 2.** Comparison of the mean changes in physiological recovery markers including (Cortisol ( $\mu\text{g/dl}$ ), IGF-1 (ng/ml), and IGF-1/Cortisol) in wrestlers in the EXE+S and EXE+P groups at different times of the simulated wrestling protocol.

Variables		EXE+S (n=10) Mean $\pm$ SD	EXE+P (n=10) Mean $\pm$ SD	P-Value
Cortisol ( $\mu\text{g/dl}$ )	B1	4.84 $\pm$ 1.23	4.71 $\pm$ 1.10	0.79
	B2	3.79 $\pm$ 1.04	5.62 $\pm$ 2.57	0.006*
	B3	5.48 $\pm$ 1.96	10.37 $\pm$ 3.69	0.002*
	B4	7.37 $\pm$ 3.48	18.45 $\pm$ 10.76	0.008*
	B5	7.76 $\pm$ 3.64	20.01 $\pm$ 9.55	0.002*
IGF-1 (ng/ml)	B1	227.04 $\pm$ 40.78	202.37 $\pm$ 47.44	0.23
	B2	280.37 $\pm$ 67.49	188.65 $\pm$ 51.51	0.001*
	B3	264 $\pm$ 57.22	188.32 $\pm$ 26.82	0.004*
	B4	230.31 $\pm$ 49.41	187.72 $\pm$ 25.23	0.04*
	B5	230.64 $\pm$ 43.12	172.23 $\pm$ 47.52	0.013*
IGF-1/cortisol	B1	4.89 $\pm$ 1.27	4.48 $\pm$ 1.47	0.51
	B2	7.83 $\pm$ 2.57	3.76 $\pm$ 1.47	0.001*
	B3	5.41 $\pm$ 2.15	1.99 $\pm$ 0.66	0.001*
	B4	4.02 $\pm$ 2.35	1.27 $\pm$ 0.55	0.003*
	B5	3.47 $\pm$ 1.61	0.99 $\pm$ 0.46	0.001*

**Note:** Significance at the level of  $\alpha=0.05$ , SD: Standard Deviation, IGF-1: Insulin-like Growth Factor-1, IGF/COR: Ratio of Insulin-like Growth Factor-1 to Cortisol, B1: Blood sampling before supplementation, B2: Blood sampling after supplementation, B3: Blood sampling after the first repetition of the wrestling-specific protocol, B4: Blood sampling after the third repetition of the wrestling-specific protocol, B5: Blood sampling after the fifth repetition of the wrestling-specific protocol.



**Figure 3:** Mean changes of perceived recovery status in EXE+S and EXE+P groups, (0-10).

In addition, in the EXE+S group, immediately after the first repetition of the specific exercise (EXE+S=(-45.47%), EXE+P=(+1.44%);  $p=0.03$ ) and immediately after the second repetition of the specific exercise (EXE+S=(-45.47%), EXE+P=(+1.44%);  $p=0.01$ ), urinary creatinine levels showed a significant decrease compared to the EXE+P group ( $P<0.05$ ). In other steps of the simulated wrestling protocol, urinary creatinine levels did not show a significant decrease compared to the EXE+P group ( $P<0.05$ ) (Table 3). 3-MH, in the EXE+S group compared to the EXE+P group, in steps after the taking HMB-FA supplement (EXE+S=(-10.46%), EXE+P=(+78.09%);  $p=0.001$ ), immediately after the second (EXE+S=(-4.8%), EXE+P=(+98.7%);  $p=0.002$ ), third (EXE+S

=(-8.16%), EXE+P=(+113.11%);  $p=0.001$ ), fourth (EXE+S=(+20.07%), EXE+P=(+113.42%);  $p=0.01$ ) and fifth (EXE+S=(+11.3%), EXE+P=(+127.06%);  $p=0.001$ ) repetitions of specific exercise showed a significant decrease ( $P<0.05$ ).

3-MH/Cr in the EXE+S group compared to the EXE+P group, in the steps after the taking HMB-FA supplement (EXE+S=(-45.47%), EXE+P=(+1.44%);  $p=0.001$ ), immediately after the second (EXE+S=(-46.8%), EXE+P=(-0.62%);  $p=0.007$ ), the third (EXE+S=(-55.14%), EXE+P=(-1.38%);  $p=0.001$ ), the fourth (EXE+S=(-45.84%), EXE+P=(+1.39%);  $p=0.001$ ) and the fifth (EXE+S=(-48.06%), EXE+P=(+2.8%);  $p=0.001$ ) repetitions of specific exercise showed a significant decrease ( $P<0.05$ ) (Table 3). In the EXE+S group, compared to the EXE+P group, LDH had a significant decrease in steps immediately after the first (EXE+S=(+9.58%), EXE+P=(+47.6%);  $p=0.005$ ), the third (EXE+S=(+10.44%), EXE+P=(+59.62);  $p=0.002$ ) and the fifth (EXE+S=(+14.35%), EXE+P=(+58.34%);  $p=0.001$ ) repetitions of specific exercise ( $P<0.05$ ). CK showed a significant decrease in the EXE+S group compared to the EXE+P group only in the step immediately after the fifth repetition (EXE+S=(+95.38%), EXE+P=(+246.44%);  $p=0.04$ ) of a specific exercise ( $P<0.05$ ).

**Table 3.** Comparison of the mean changes in physiological recovery markers (including urinary Cr (mg/ml), urinary 3-MH ( $\mu\text{mol/ml}$ ), urinary 3-MH/Cr (nmol/mg)), LDH (U/L), and CK (U/L)) in wrestlers in the EXE+S and EXE+P groups at different times of the simulated wrestling protocol.

Variables		EXE+S (n=10) Mean $\pm$ SD	EXE+P (n=10) Mean $\pm$ SD	P-Value
Cr (mg/ml)	U1	1.70 $\pm$ 0.40	1.80 $\pm$ 0.40	0.49
	U2	2.63 $\pm$ 2.20	1.93 $\pm$ 0.2	0.39
	U3	2.63 $\pm$ 0.84	3.68 $\pm$ 1.8	0.03*
	U4	3.27 $\pm$ 1.84	3.73 $\pm$ 1.42	0.01*
	U5	3.76 $\pm$ 1.24	4.3 $\pm$ 1.95	0.49
	U6	3.06 $\pm$ 1.41	3.91 $\pm$ 1.54	0.16
	U7	3.08 $\pm$ 1.80	3.83 $\pm$ 1.25	0.37
3-MH ( $\mu\text{mol/ml}$ )	U1	181.66 $\pm$ 19.08	189.72 $\pm$ 21.71	0.51
	U2	162.65 $\pm$ 55.61	337.89 $\pm$ 71.85	0.001*
	U3	201.55 $\pm$ 91.72	357.69 $\pm$ 77.61	0.07
	U4	172.94 $\pm$ 55.45	376.93 $\pm$ 62.42	0.002*
	U5	166.82 $\pm$ 55.82	404.32 $\pm$ 44.27	0.001*
	U6	218.12 $\pm$ 94.30	404.91 $\pm$ 46.97	0.01*
	U7	202.2 $\pm$ 70.74	430.79 $\pm$ 28.49	0.001*
3-MH/Cr (nmol/mg)	U1	133.85 $\pm$ 9.09	136.11 $\pm$ 9.82	0.6
	U2	72.98 $\pm$ 17.39	138.08 $\pm$ 38.44	0.001*
	U3	82.44 $\pm$ 42.64	125.08 $\pm$ 50.78	0.06
	U4	71.20 $\pm$ 35.39	135.26 $\pm$ 57.84	0.007*
	U5	60.04 $\pm$ 30.05	134.23 $\pm$ 47.71	0.001*
	U6	72.48 $\pm$ 31.46	138.01 $\pm$ 31.96	0.001*
	U7	69.51 $\pm$ 22.68	139.93 $\pm$ 32.44	0.001*
LDH (U/L)	B1	358.9 $\pm$ 70.30	367.8 $\pm$ 27.46	0.2
	B2	345.6 $\pm$ 40.53	370 $\pm$ 26.23	0.105



	B3	393.3 ± 74.03	542.9 ± 121.45	0.005*
	B4	396.4 ± 76.35	587.1 ± 131.24	0.002*
	B5	410.4 ± 53.52	582.4 ± 83.24	0.001*
CPK (U/L)	B1	130.10 ± 25.23	119.70 ± 31.86	0.31
	B2	113 ± 21.84	129.90 ± 23.84	0.14
	B3	231.6 ± 98.75	327.50 ± 175.31	0.19
	B4	268.20 ± 99.60	367.30 ± 206.72	0.23
	B5	254.20 ± 90.11	414.70 ± 186.92	0.04*

**Note:** Significance at the level of  $\alpha=0.05$ , Cr: Creatinine, 3-MH: 3-Methylhistidine, 3-MH/Cr: 3-Methylhistidine/Creatinine ratio, LDH: Lactate Dehydrogenase, CK: Creatine Kinase, B1: Blood sampling before supplementation, B2: Blood sampling after supplementation, B3: Blood sampling after the first repetition of the wrestling specific protocol, B4: Blood sampling after the third repetition of the wrestling specific protocol, B5: Blood sampling after the fifth repetition of the wrestling specific protocol, U1: Urine collection before supplementation, U2: Urine collection after supplementation, U3: Urine collection after the first repetition of the specific wrestling protocol, U4: Urine collection after the second repetition of the specific wrestling protocol, U5: Urine collection after the third repetition wrestling specific protocol, U6: Urine collection after the fourth repetition of the wrestling specific protocol, U7: Urine collection after the fifth repetition of the wrestling specific protocol.

## Discussion

The main findings of our study revealed that HMB-FA supplementation improved physiological recovery markers such as cortisol, IGF-1, IGF-1/cortisol ratios (Table 2) and perceived recovery state (Figure 3), and it also reduces other physiological recovery markers such as Cr, 3-MH, 3-MH/Cr, LDH and CK in elite wrestlers (Table 3).

Consistent with previous research involving athletic populations, HMB-FA supplementation promoted muscle recovery, reduced muscle injury, increased intramuscular anabolic signaling, and improved exercise performance. In the present study, there were significant reduction in cortisol, and significant increase in Insulin-like Growth Factor-1 (IGF-1) and IGF-1/cortisol ratio in the EXE+S group. In several studies, HMB-FA supplementation has been shown to increase anabolic hormones (GH and IGF-1) and reduce catabolic hormones (CORT and ACTH), and it appears that HMB-FA can modify the GH/IGF-1 axis without altering circulating testosterone levels. Our findings support and coincide with these studies [18].

On the other, the Cr, 3-MH, 3-MH/Cr, LDH, and CK decreased in the EXE+S group. The ratios of Cr, 3-MH, and 3-MH/Cr increased in the EXE+P group, indicating muscle protein breakdown. Various studies have previously shown serum CK and LDH as indices of skeletal muscle damage and urinary 3-MH and 3-MH/Cr ratios as indicators of protein breakdown. Short-term HMB-FA supplementation has been shown to decrease muscle damage indices after a high-volume resistance training session. Muscle protein breakdown, measured by the urinary 3-methylhistidine/creatinine ratio, remained constant in the placebo group, while approaching a statistically lower ratio in the HMB-FA group 48 hours after exercise. Therefore, HMB-FA supplementation appeared to mitigate resistance exercise-induced muscle damage and improve recovery speed in trained men. Our findings support and coincide with these studies. However, it is essential to acknowledge differences in study designs, populations, and specific outcomes evaluated in these studies. Consequently, a direct comparison may only be somewhat appropriate and more research is needed to better understand the precise mechanisms underlying the observed benefits of HMB-FA supplementation in wrestlers.

Potential mechanisms responsible for the observed improvements in protein balance and recovery indices could include the role of HMB-FA in reducing muscle protein breakdown, increasing muscle protein synthesis, and mitigating muscle damage. Accelerated absorption of HMB-FA, compared to other forms of

HMB, may have contributed to the positive outcomes observed in this study. HMB-FA has been shown to result in a net positive balance of protein turnover from skeletal muscle by stimulating protein synthesis and attenuating protein degradation. HMB-FA stimulates protein synthesis through changes in GH/IGF-1 axis activity. Other mechanisms of action of HMB-FA include inhibition and increased proteolysis and protein synthesis. HMB-FA appears to stimulate muscle protein synthesis through up-regulation of the mammalian/mechanical target of rapamycin complex 1, a signaling cascade involved in the coordination of translation initiation of muscle protein synthesis. Furthermore, HMB-FA may have antagonistic effects on the ubiquitin proteasome pathway, a system that degrades intracellular proteins. Evidence also suggests that HMB-FA promotes myogenic proliferation, differentiation, and cell fusion. Therefore, in addition to its effects on the ubiquitin proteasome pathway, HMB-FA may also attenuate the degradation of skeletal muscle proteins by inhibiting caspase activity [19].

In the present study, the EXE+P group exhibited a reduction in PRS score after the steps of the wrestling specific protocol. However, the EXE+S group had a higher score on the perceived recovery scale. The decrease in PRS in the EXE+P group appears to be due to an increase in cortisol, a reduction in the IGF-1 and IGF-1/cortisol ratio, and an increase in serum LDH and CK. However, to our knowledge, no such finding has been reported so far on the perceived recovery status of wrestlers in a simulated wrestling protocol.

Despite promising results, our study has several limitations that warrant consideration. First, the small sample size may limit the generalizability of our findings to the larger wrestler population. Second, possible confounding factors, such as individual differences in training status, nutritional habits, and genetic predispositions, could have influenced the results. Third, the use of a simulated wrestling protocol instead of real competition could have impacted the external validity of our findings. Future research should address these limitations by including larger sample sizes, controlling for potential confounders, and incorporating real competition scenarios [20].

## Conclusion

In conclusion, our findings suggest that HMB-FA supplementation may increase intramuscular anabolic signaling, stimulate muscle protein synthesis, reduce muscle protein breakdown, attenuate markers of muscle damage, augment acute endocrine responses,

and finally, increases the speed of recovery following a simulated wrestling protocol in elite wrestlers. Although more research is needed to confirm these results and understand the precise mechanisms involved, HMB-FA supplementation could potentially serve as a valuable tool for wrestlers and other athletes to improve their performance and recovery.

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## Author Contributions

Conceptualization, B.T, B.R.; methodology, B.T, B.R.; software, B.T, B.R.; validation, B.T, B.R.; investigation, B.T, B.R. resources, B.T, B.R.; formal analysis, B.T, B.R.; data curation, B.T, B.R.; writing original draft preparation, B.T, B.R.; review and editing the first draft, B.T, B.R.; project administration, B.T, B.R.; funding acquisition, B.T, B.R. All authors have read and agreed to the published version of the manuscript.

## Competing Interests

The authors declare no competing interests.

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