



## Short-Term HMB Supplementation Reduces Muscle Damage after a Bout of Resistance Training in non-Athletic Females

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### Abstract

Starting or continuing physical activity, especially for non-athletes, can be a challenge due to muscle injuries caused by physical activity. Therefore, the present study aimed to investigate the short-term effect of beta-hydroxy beta-methyl butyrate (HMB) supplementation on muscle and liver damage caused by a period of resistance activity in non-athletic females. Among the volunteers, 16 female non-athletes with an average age of  $21.75 \pm 1.18$  years, a body mass index of  $24.83 \pm 2.67$  kg/m<sup>2</sup>, and a weight of  $63.43 \pm 8.46$  kg were randomly selected as a statistical sample. The subjects were randomly divided into two groups of eight people, HMB supplement and placebo. Daily and for six days, the subjects of the supplement group received 3 mg of beta-hydroxy beta-methyl butyrate powder. The placebo group received 3 grams of starch in tablet form. After six days of loading, the subjects performed a resistance activity session with an intensity of 75-80% 1RM. Blood samples were taken in five stages, including before supplementation, before training, immediately, 1 hour, and 24 hours after sports activity. To compare the results, a 5x2 analysis of variance test was used. The results showed that the consumption of HMB supplements significantly affected the serum levels of Creatine kinase (CK) and lactate dehydrogenase (LDH) enzymes in the blood and the amount of aspartate aminotransferase (AST) and alanine aminotransferase enzyme activity in the blood of intragroup interactions ( $p < 0.05$ ). On the other hand, there was no significant difference between the serum levels of CK enzyme and the activity of ALT and AST enzymes between the two supplement and placebo groups ( $p > 0.05$ ). There was no significant difference in the serum level of LDH enzyme between the two supplement and placebo groups ( $P < 0.05$ ). Although the results of the present study showed that consuming 3 grams of HMB supplement reduces the LDH response after resistance training, this supplement cannot be used as an independent factor for reducing muscle damage markers following intense physical activities. As a new achievement, it is recommended that HMB supplements be taken more cautiously to reduce indicators of muscle damage.

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## INTRODUCTION

The most important and effective way to increase the ability and reach the peak of fitness is a combination of practical exercises and proper nutrition (Mujika et al., 2018). Muscle tissue may be damaged after intense and long-term training due to metabolic and mechanical factors (Schoenfeld, 2013). Intense muscle training frequently causes muscle damage, which is defined by ultrastructural changes in the muscle tissue, as well as clinical symptoms such as a reduction in muscle power and range of motion, as well as an increase in pain and edema. On the other hand, the liver, the largest "chemical organ" in the human body, is involved in critical biological processes such as metabolism, digestion, and detoxification, which are essential for maintaining health (Fu et al., 2022). The serum level of enzymes or proteins in skeletal muscle and liver indicates the functional state of muscle tissue and is very different in pathological and physiological conditions (Kaur et al., 2019). CK and LDH are the most useful serum markers of muscle damage that may change after intense physical activity (Fernández-Landa et al., 2020). As complex metabolic cells, liver cells contain high amounts of enzymes, which can be used to detect liver damage in time by detecting changes in serum enzymes (Liu et al., 2017). Cytoplasmic enzymes AST and ALT are the main indicators of liver cell damage (Paddon-Jones et al., 2001). Since these enzymes were considered as an indirect indicator of muscle damage and when muscle damage occurs, they are increased in the blood, which is located in the muscle fibers (Paddon-Jones et al., 2001). Since exercise and supplementation are important in therapeutic approaches (Hoseini et al., 2022a; Hosseini et al., 2021), Many studies have investigated the effectiveness of pharmaceutical and nutritional interventions to reduce the signs and symptoms of exercise-induced muscle damage, with mixed results (Hoseini et al., 2022b). According to new research, using some supplements can have constructive and useful effects in preventing the complications of intense exercises (Hossain et al., 2019; Hosseini et al., 2021). An example of these food supplements is HMB, a prerequisite amino acid for leucine production (Holeček, 2017). The anti-catabolic property of leucine has been known for years (Wilkinson et al., 2013). Leucine is transformed into ketoisocaproate (KIC) by the enzyme KIC dioxygenase after entering the body. KIC is then transformed into isovaleryl CoA in the mitochondria by the enzyme alpha-factoid-dehydrogenase. In the cytosol, it is transformed into HMB by the enzyme alpha-factoisocaproate deoxygenase (Ananieva et al., 2016).

HMB supplement facilitates the recovery capacity of skeletal muscle after intense exercise and increases its speed. In addition, it has been shown that HMB supplement stimulates muscle protein synthesis and reduces markers of muscle damage and muscle protein breakdown (Kaczka et al., 2019). Regarding the effective amount of HMB, many studies have shown that HMB supplements with an amount of 1.5 to 3 grams, approximately equal to 38.1 mg per kilogram of body weight, have a pleasing effect in reducing muscle protein breakdown and increasing strength and muscle mass after resistance training (De Pinho et al., 2019), in a report consisting of several studies, showed that consuming 1.5, 0.5, and 3 grams of HMB per day along with 1.5 to 3 hours of weight training per week for three weeks (first study) and consuming 0 and 3 grams of HMB per day along with 2-3 hours of weight training, six days a week for seven weeks (second study) reduces the indices of proteolysis and muscle damage (urinary 3-methylhistidine and serum creatine phosphokinase) caused by stimulation (Nissen et al., 2003). It becomes mechanical and increases muscle mass (Nissen et al., 2003). In 2007, Muller investigated the effect of HMB supplementation on body composition and muscle production capacity in non-competitive male athletes aged 19 to 24 years who performed resistance training three times a week for eight weeks. This study showed decreased CK activity (Muller et al., 2007). Did not confirm the effectiveness of this supplement in a study (Slater et al., 2001). Showed that short-term consumption of HMB at the rate of 40 mg/kg of body weight per day for six days before a session of intense isokinetic contractions has no significant effect on muscle damage factors caused by extrinsic contractions (Paddon-Jones et al., 2001).

Since few studies have investigated the effect of this new supplement on preventing muscle damage caused by short-term resistance sports activities, there is a conflict in the serum concentration of studied enzymes, especially with the HMB supplement. In some cases, reduction or stabilization of muscle damage indicators (Arazi et al., 2018), and others have reported their increase (Knechtel et al., 2011).

## METHOD

### Experimental design

This study had a randomized, double-blind, placebo-controlled design. All the subjects were familiarized with the training program two weeks before the start of the study. One week later, the subjects were again taken to the laboratory for age, height, and weight assessment. To prescribe a resistance training program, one maximum repetition (1RM) in each exercise on two non-consecutive days, which included: leg press, bench press, knee extension, biceps curl, knee flexion, shoulder press, and triceps muscles, was evaluated. To prescribe a resistance training program, one maximum repetition in each exercise on two non-consecutive days: leg press, bench press, knee extension, biceps curl, knee flexion, shoulder press, and triceps muscles, was evaluated (Cawthorn et al., 2014).

### Participants

In this research, 16 healthy non-athlete females aged 20-24 were selected to participate. To be included in the final analyses, subjects had to complete all training sessions and participate in all assessment sessions. As a result of these requirements, all 16 people participated in the study until the end. Therefore, 16 non-athlete females were included in the final analysis. Participants were divided into two study groups, HMB and Placebo, with anthropometric and physical characteristics as described (Table 1). The subjects had the following criteria for entering the study: (1) non-athlete females with no history of regular training in the past year. (2) Subjects did not use any medicinal or nutritional supplements during the research period (3) 6 months with no musculoskeletal injury or any orthopedic problem that could affect the efficiency of resistance training. (4) During the intervention phase, refrain from engaging in any competitive sports activity other than resistance training. In addition, the subjects were informed in their training program with experimental methods and possible risks and benefits related to participating in the study. Before starting the study, all subjects completed the health questionnaire and consent form. Their nutrition was followed normally and habitually.

**Table.1-** Anthropometric and physical characteristics of the participants

### Measurements

Group		Age	Height	Weight	BMI
HMB	Mean	21.3750±0.7440	159.8750±4.7939	64.1250± 10.2878	25.0313± 3.4761
	N	8	8	8	8
Placibo	Mean	22.1250±1.4577	159.3750±4.2067	62.7500± 6.84001	24.6437± 1.7736
	N	8	8	8	8

The subjects' height was measured using a wall-mounted stadiometer (Seca 222, Terre Haute, IN, USA) with an accuracy of 0.5 cm. Body weight was measured using a digital scale (Tanita, BC-418MA, Tokyo, Japan) with an accuracy of 0.1 kg, and body mass index (kg/m<sup>2</sup>) was calculated.

### Blood sampling

One week before the test, blood samples were taken to determine the baseline levels of CK, LDH, AST, and ALT in the amount of 8 ccs from the brachial vein and after 10 hours of fasting in a sitting position. The subjects were asked to refrain from intense sports activities and heavy work for two days before the first stage of blood sampling and then during the research. After six days of taking the supplement and placebo, the subjects appeared on the morning of the test at 8 o'clock fasting, and the second blood sample was taken from the brachial vein to measure the values of CK, LDH, AST, and ALT indices (pre-test). Immediately after the second blood sampling and (one hour) after having a standard breakfast (including wheat bread, butter, and jam containing approximately 300 kilocalories), the subjects performed the exercise protocol. Immediately, 1 hour and 24 hours after the training protocol, the third, fourth, and fifth blood samples were taken as (post-test) in two stages. Blood sampling was done after 10 hours of fasting between 8 and 9 in the morning, in a sitting position, in the amount of 5 ccs from the brachial vein. Blood samples were centrifuged at 3000 rpm

for 15 minutes, and serum after coding was stored in a special rack in 3 ml microtubes at -70 degrees Celsius.

#### *Biochemical analysis*

Serum samples (LDH-CK) were analyzed using commercial enzyme-linked immunosorbent assay (ELISA) kits (ZellBio GmbH Veltlinerweg 29, 890, Germany). The activity of alanine transaminase ALT and aspartate aminotransferase AST was measured using an automatic analyzer (model 747-400, Hitachi, Tokyo, Japan). All assays were performed in duplicate. The coefficient of variation for the measurements was less than 7%.

#### *Supplement*

After taking the measurements and without informing the subjects, they were randomly divided into a supplement group HMB and a placebo group. They were told to consume 3 grams of the received supplements in the form of (500 mg capsules) daily for six days in three meals (breakfast, lunch, and dinner) (Jówko et al., 2001). The HMB supplement contained 3 g of beta-hydroxy beta-methyl butyrate in powder form per serving (BetaTor, Body Attack, GmbH & Co., KG, Waldhofstrabe 19, 25474, Ellerbeck, Germany). Each serving of the placebo contained 3 grams of starch. Also, all the subjects of the two groups were requested to follow their usual diet during the study, not to change their physical activity, or not participate in other high-intensity sports activities. Without letting the subjects know what kind of supplement they were to take, the researcher had already manufactured the HMB supplement and a placebo in cone-shaped capsules. To ensure the consumption of pills, the subjects were reminded daily to use the SMS system. The subjects were also asked to return all used and unused packets to the researcher at the end of 6 days of use.

#### *Exercise programs*

In this study, the exercise protocol included the implementation of 7 movements: chest press (barbell), back of the arm (barbell), front shoulder press (barbell), front arm (barbell), underarm barbell, front leg and back leg (machine), which was considered in 3 sets and eight repetitions with rest intervals of (2-3) minutes between each movement and (30-90) seconds between sets. It should be mentioned that the exercise program used in this study has already been used in other studies to induce muscle damage (Cooke et al., 2010). All movements were performed under the supervision of researchers in the physical training hall of the Faculty of Physical Education and Sports Sciences of Halabja University. Before starting the plan, in a preliminary meeting, while explaining the plan's goals, a maximum repetition test (1RM) was performed on all the above movements so that resistance activity based on 75-80% of a maximum repetition was performed. A maximum repetition test was performed based on Berzyski's formula:

$1RM = \{Number\ of\ repetitions / 30 + 1\} \times Weight\ used$  Subjects warmed up with stretching exercises (general warm-up) for 10-12 minutes each before starting the training protocol. At the end of each resistance activity session, the subjects were asked to cool down their body with gentle stretching movements. It should be mentioned that the exercise program used in this study has already been used in other studies to induce muscle damage (Cooke et al., 2010). All movements were performed under the supervision of the researcher and her colleagues.

#### *Statistical Analysis*

Data were presented as mean  $\pm$  standard deviation. The distribution of each variable was analyzed using the Shapiro-Wilk test. Levine's test was used to examine the homogeneity of variances and analysis of variance of repeated measurements to explore the difference between different sampling times. Bonferroni's follow-up test was used. All analyzes were performed using SPSS 26.0 (SPSS Inc., Chicago, Ill., USA) and GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA, USA). The level of statistical significance was determined as P-value  $\leq$  0.05.

## RESULTS AND DISCUSSION

### Results

The physical and anthropometric characteristics of the participants are presented in (Table 2). No significant difference was observed between the average of the HMB supplement and placebo groups in any of the mentioned indicators.

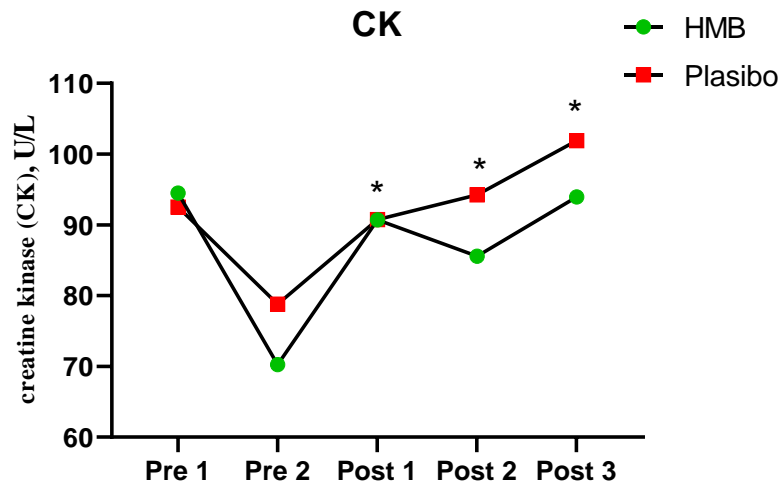
**Table 2.** Subject characteristics

Group		Age	Height	Weight	BMI
HMB	Mean	21.3750	159.8750	64.1250	25.0313
	Std. Deviation	.74402	4.79397	10.28782	3.47615
Placibo	Mean	22.1250	159.3750	62.7500	24.6437
	Std. Deviation	1.45774	4.20671	6.84001	1.77364
Total	Mean	21.7500	159.6250	63.4375	24.8375
	Std. Deviation	1.18322	4.36463	8.46931	2.67341

### *The effect of HMB supplementation on the reduction of blood serum creatine kinase (CK) levels*

To observe intra-group changes in each group, repeated one-way analysis of variance and Bonferroni post hoc test were used, and differences between the two groups at different times from the training protocol, independent t-test with alpha adjustment of 0.0125 were used. The mean and standard deviation of the changes in serum CK blood levels of the subjects in 5 stages of the test: pre-test 1 (before starting to take the supplement), pre-test 2 (before the exercise protocol), post-test 1 (immediately after the exercise protocol), post-test 2 (1 hour after the training protocol) and post-test 3 (24 hours after the training protocol) were examined.

The results of the analysis of the variance test showed that the changes in group age were significant in the interaction with time ( $F=4.814$ ;  $p=0.015$ ) but not significant in the interaction with (time  $\times$  group) ( $F=0.414$ ;  $p=0.673$ ). The results of Bonferroni's post hoc test showed a significant difference between the average of the pre-test 2 group and post-test 1 ( $p=0.001$ ), post-test 2 ( $p=0.001$ ), and post-test three groups ( $p=0.031$ ). No significant difference was observed between other groups ( $p>0.05$ ). The results of the Independent-Samples T-Test showed an increase between the HMB supplement group and placebo in pre-test 1 (2.11%). Still, in pre-test 2 (-10.87%), post-test 1 (-0.08%), post-test 2 (-9.23%), and post-test 3 (-7.83%), a decrease in CK concentration was observed, but the difference was not significant ( $p>0.05$ ; see Figure 1).



**Figure 1.** The effect of HMB supplementation on reducing blood serum creatine kinase (CK) levels

Data are represented as mean  $\pm$  SEM.

**Abbreviations:** **Pre 1:** Before starting the supplement- **Pre 2:** Before the training protocol

**Post 1:** Immediately after the training protocol **Post 2:** 1 hour after the training protocol

**Post 3:** 24 hours after the training protocol

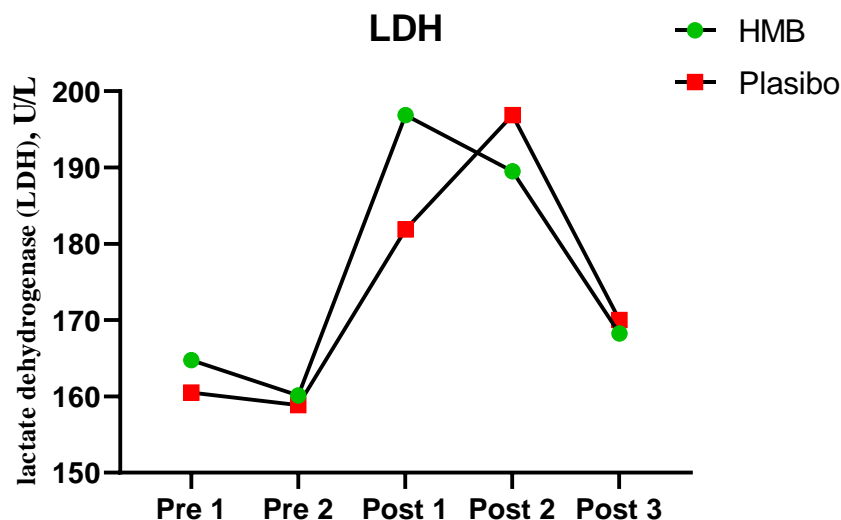
**Repeated measurement:** \*vs Pre 2 ( $P < 0.05$ ).

**Independent-Samples T Test:** ( $p > 0.05$ )

#### *The effect of HMB supplementation on reducing serum lactate dehydrogenase (LDH) levels*

The statistical analysis of the data shows that, in general, there is no significant difference between time and group (time  $\times$  group interaction) ( $F=1.450$ ,  $p=0.906$ ). By examining intra-group changes, time had no significant effect on LDH serum concentration in any test stages ( $F=2.450$ ,  $p=0.080$ ). Despite the average difference (-2.80%) in the reduction of LDH concentration between pre-test 2 and 1 and the average difference between post-test 2 and 3 compared to post-test one by (-3.72, -14.53) in the HMB supplement group, the mean difference was not significant in any of them ( $p > 0.05$ ). Examining the intra-group difference in the HMB supplement group showed that despite the average difference in the post-test stages 1, 2, and 3 compared to the pre-test 2 in the amount of (22.95%, 18.34%, 5.07%) respectively, the increase in LDH serum concentration in The level was not significant ( $p > 0.05$ ). The results of the Independent-Samples T-Test showed that between pre-test 1 ( $t_{14}=0.223$ ,  $p=0.827$ ) and pre-test 2 ( $t_{14}=0.51$ ,  $p=0.960$ ) and post-test ( $t_{14}=0.602$ ,  $p=0.557$ ) to the extent of (-2.57%, -0.87%, -7.61%) in the two HMB supplement groups, respectively, compared to the placebo group, a decrease in LDH concentration was observed, but the difference in means was not significant ( $p > 0.05$ ; see [Figure 2](#)).





**Figure 2.** The effect of HMB supplementation on reducing serum lactate dehydrogenase (LDH) levels

Data are represented as mean  $\pm$  SEM.

**Abbreviations:** **Pre 1:** Before starting the supplement- **Pre 2:** Before the training protocol

**Post 1:** Immediately after the training protocol **Post 2:** 1 hour after the training protocol

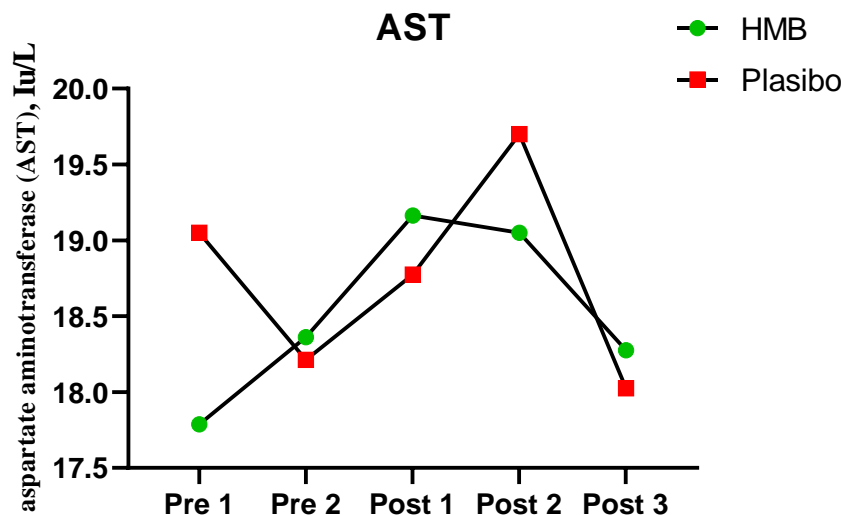
**Post 3:** 24 hours after the training protocol

**Repeated measurement:** ( $p > 0.05$ )

**Independent-Samples T Test:** ( $P > 0.05$ ).

#### *The effect of HMB supplementation on reducing serum aspartate aminotransferase (AST) levels*

The analysis of the variance test showed that the changes in group period in interaction with time ( $F=636$ ;  $p=0.603$ ) and Time  $\times$  Group ( $F=299$ ;  $p=0.803$ ) were insignificant. In the analysis of intra-group changes, it was discovered that in the HMB supplement group, AST activity in the blood increased by 3.26% and 7.76%, respectively, in the pre-test two and post-test one stages compared to the pre-test 1, although these changes were not statistically significant ( $p > 0.05$ ). In the comparison of the HMB supplement group, in the 2nd and 3rd post-test stages compared to the 1st post-test, a decrease in AST activity in the blood was observed -0.57%, -4.64%, respectively, but the changes were not significant ( $p > 0.05$ ). In the placebo group, in the stages of pre-test two and post-test 3, compared to pre-test 1, AST concentration decreased by -4.40%, -5.40%, respectively, but it was not significant ( $p > 0.05$ ). In the placebo group, in the post-test stages 1 and 2 compared to the pre-test 2, the AST concentration increased by 3.07% and 3.75%, respectively, but the mean difference was insignificant ( $p > 0.05$ ). In examining inter-group changes using the T-test between the two groups of HMB supplement and placebo, no significant changes were observed in any of the test stages of the two groups ( $p > 0.05$ ). The average difference between the two groups of HMB supplement and placebo in the steps of pre-test 1 ( $t_{14}=0.749$ ,  $p=0.466$ ) and post-test 2 ( $t_{14}=0.706$ ,  $p=0.492$ ) is -6.66%, -3.29%, respectively, decreasing the sign but the changes were not significant ( $p > 0.05$ ). The average difference between the two groups of HMB supplement and placebo in the stages of pre-test 2 ( $t_{14}=0.138$ ,  $p=0.892$ ), post-test 1 ( $t_{14}=0.311$ ,  $p=0.760$ ), and post-test 3 ( $t_{14}=0.134$ ,  $p=0.895$ ) is as much as 0.82%, 2.07, 1.38% showed an increase respectively, but the changes were not significant ( $p > 0.05$ ; see Figure 3). From the results, it can be concluded that probably HMB supplement will not have a positive effect on reducing the activity of AST enzyme either before the training protocol or after the training protocol.



**Figure 3.** The effect of HMB supplementation on reducing serum aspartate aminotransferase (AST) levels

Data are represented as mean  $\pm$  SEM.

**Abbreviations:** **Pre 1:** Before starting the supplement- **Pre 2:** Before the training protocol

**Post 1:** Immediately after the training protocol **Post 2:** 1 hour after the training protocol

**Post 3:** 24 hours after the training protocol

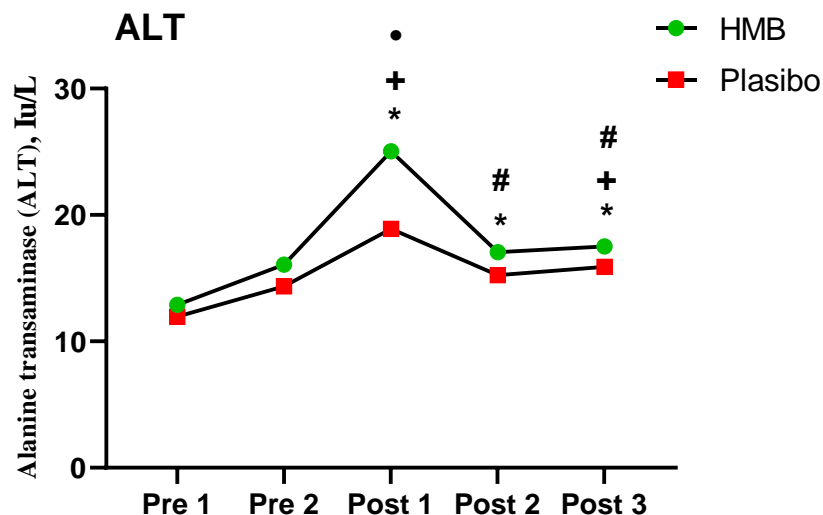
**Repeated measurement:** ( $p > 0.05$ )

**Independent-Samples T Test:** ( $P > 0.05$ ).

#### *The effect of HMB supplementation on reducing serum alanine transaminase (ALT) levels*

The analysis of the variance test showed that the changes in group period in interaction with time ( $F=35.97$ ;  $p=0.001$ ) and Time  $\times$  Group ( $F=3.285$ ;  $p=0.036$ ) were significant. In the analysis of the alterations within the group, it was discovered that the HMB supplement group experienced an increase in ALT activity in the blood in post-test stages 1, 2, and 3 compared to pre-test stage 1 by 94.18%, 32.24%, and 35.65%, respectively. The level of changes was significant ( $p < 0.05$ ). In the comparison of the HMB supplement group, in the post-test stages, 1 and 3 compared to the pre-test 2, an increase in ALT activity in the blood was observed at 55.97% and 8.96%, respectively, and the changes were significant ( $p < 0.05$ ). In comparing the HMB supplement group in the 2nd and 3rd post-test stages to the 1st post-test, a decrease in ALT activity in the blood was observed -31.89% -30.13%, respectively, and the changes were significant ( $p < 0.05$ ). In examining inter-group changes using a T-test between the two groups of HMB supplement and placebo, significant differences were observed between the two groups in the post-test stage 1 ( $t_{14}=2.896$ ,  $p=0.012$ ), significant changes were observed in the amount of (32.46%). In the steps of pre-test 1 ( $t_{14}=0.491$ ,  $p=0.631$ ), pre-test 2 ( $t_{14}=0.896$ ,  $p=0.385$ ), post-test 2 ( $t_{14}=0.909$ ,  $p=0.379$ ), and post-test 3 ( $t_{14}=0.831$ ,  $p=0.420$ ) to the extent of 7.87%, 11.91%, 12.01%, 10.06 respectively, an increase in concentration ALT was observed in the supplement HMB group, but the difference in means was not significant ( $p > 0.05$ ; see [Figure 4](#)).





**Figure 4.** The effect of HMB supplementation on reducing serum Alanine transaminase (ALT) levels

Data are represented as mean  $\pm$  SEM.

**Abbreviations:** **Pre 1:** Before starting the supplement- **Pre 2:** Before the training protocol

**Post 1:** Immediately after the training protocol **Post 2:** 1 hour after the training protocol

**Post 3:** 24 hours after the training protocol

**Repeated measurement:** \*vs Pre1, +vs Pre2, #vs Post1 ( $p > 0.05$ )

**Independent-Samples T Test:** •Post 1 ( $P > 0.05$ ).

## Discussion

Research has shown that repetitive and intense exercises, especially those extroverted movements, are usually associated with disruption and damage to the skeletal muscle's connective tissue or contractile tissue (Ely, 2019). Numerous attempts have been undertaken to identify methods to lessen exercise-induced muscle protein breakdown and promote protein synthesis since reduced muscular performance frequently accompanies skeletal muscle injury. Therefore, there is no direct evidence that any drug treatment can prevent exercise-induced muscle damage or accelerate the recovery process of damaged muscles after exercise (Kashef M, 2013).

The results of the present study showed that six days of HMB supplementation had a significant effect on the CK index at different times of the study within the group. Still, no significant changes were observed in both the HMB and the placebo groups (Figure 1). Also, no significant difference was observed in the serum levels (LDH) between the groups. Within the subjects in any of the different times of this Research (Figure 2). Based on the findings of this research and regarding the effect of HMB supplementation on CK and LDH enzymes (selective index of muscle damage), Hoffman et al., the effect of consuming 3 grams of HMB on power performance using Winget's anaerobic power test and muscle damage and stress indicators in 26 football students During ten days, they checked the training. Finally, they did not observe any significant difference in the indices of muscle damage (Hoffman et al., 2004). Short-term supplementation with 3 grams of HMB could not lessen the muscle damage brought on by extroverted exercise in trained soccer players, as indicated by the CK and LDH indices. In their study, Majid Kashif et al. reported that short-term HMB supplement use did not affect the indices of muscle damage, CK, and LDH, during resistance training (Kashef M, 2013).

On the other hand, contrary to the results of the present study, Rahimi observed a significant decrease in the CK index after taking the HMB supplement (Rahimi et al., 2018). Wolsen et al. attempted to determine the specific effects of HMB supplementation on muscle damage indicators in their research but were unable to do so. Still, they stated that taking HMB supplementation before exercise prevents the increase of LDH (Wilson et al., 2013). Niter et al. have also reported the significant effect of 3 grams of HMB supplementation per day on the reduction of CK and LDH indicators of muscle damage in the HMB group compared to the placebo group (Knitter et al., 2000).

In this study, no significant changes were observed in the serum levels AST after six days of HMB supplementation and placebo at different times of the research, within and between groups ([Figure 3](#)). Although serum levels of ALT within the group at different times and among both HMB supplement and placebo groups were significant immediately after the exercise protocol, there were significant changes in the phase before taking the supplement and placebo and one and 24 hours after the exercise protocol. It was not observed. Sports activities, which are influenced by the duration, intensity, kind, and exercise method, increase the activity of liver enzymes ([Bird & Hawley, 2017](#)). As metabolically complex, liver cells contain high levels of ALT and AST enzymes. These cytoplasmic enzymes are the leading indicators of liver cell damage ([Nakagaki et al., 2018](#)). When muscle damage occurs, enzymes such as CK, LDH, AST, and ALT, located in muscle fibers and the liver, increase in the blood ([Asaikumar et al., 2019](#)). AST and ALT enzymes are abundant in the liver. AST is significant in other tissues, such as the heart, kidneys, skeletal muscles, and red blood cells. But the concentration of ALT in skeletal muscles is low ([Ismail, 2022](#)). Increased serum AST and ALT indicate the entry of muscle and liver enzymes into the blood circulation. Therefore, changing the concentration of these enzymes can cause muscle damage ([Lehmann-Werman et al., 2018](#)).

Research has been conducted regarding the effect of HMB supplementation on the liver enzyme index AST, and different results have been reported according to the type of training, preparation, and gender of the subjects. For example, Kreider et al., consistent with the results of the present study and other studies, did not observe a significant difference in the increase of AST after 28 days of HMB supplementation in soccer players ([Kreider et al., 2000](#)). On the other hand, in opposition to the results of the present study, in their research entitled "Evaluation of the dose-dependent effect of HMB supplementation on the indicators of muscle and liver damage caused by a session of eccentric resistance activity, among the doses examined research in quantities of 3 and 4 mg per day were able to prevent a significant increase in serum enzymes as indicators of liver damage.

Regarding the effect of HMB supplementation on ALT liver enzyme index, in the present study, a significant increase in ALT serum levels within the group was observed at different times and between both HMB supplementation and placebo groups immediately after the exercise protocol. Contrary to the present research results, Arazi et al. investigated the effects of 6 weeks of resistance training with the consumption of 3 grams per day of HMB-FA supplement on oxidative stress and liver enzymes. They observed a significant decrease in serum ALT levels ([Arazi et al., 2018](#)). In this study, no significant changes were observed in the phase before taking the supplement and placebo and only one and 24 hours after the exercise protocol ([Figure 4](#)). In this regard, in line with the present research, found that HMB supplementation does not have a significant effect on serum ALT levels in one and 24 hours after exercise.

Recent studies have suggested that HMB plays a role in several mechanisms, such as inhibition of muscle proteolysis, including co-stimulation of the mTOR pathway (mTOR) kinase, which directs translational precursors of protein synthesis, and inhibition of the ubiquitin-proteasome pathway (the primary regulatory system in skeletal muscle breakdown) ([J Cruz-Jentoft, 2018](#)). It is also essential to improve cholesterol metabolism, considered a prominent part of the cell membrane, not only for the integrity of the cell wall but also for regulating intracellular processes ([Nissen et al., 2000](#)). Increased cell wall remodeling capacity may reduce damage to intracellular processes that depend on this integrity. This process is critical because of its dependence on cholesterol regeneration, especially in muscle tissue. In support of this theory, known as the theory of cholesterol synthesis and increases membrane function, studies show that a delay in cholesterol synthesis impairs muscle function and leads to increased muscle damage, resulting in necrosis. Cholesterol is produced from acetyl-CoA, which is synthesized slowly from mevalonic acid, a cholesterol precursor in the cytosol of muscle and liver cells ([Nissen et al., 2000](#)). This process takes place with the intervention of the hydroxy-methyl-glutaryl reductase enzyme. Most of the HMB is converted to HMB-COA, which is then converted to cholesterol. The damaged muscle cannot produce enough cholesterol to stabilize the sarcolemma. The consumption of HMB can effectively rebuild and repair damaged cell walls during intense physical activity. This way, the release of enzymes such as CK from the cell is prevented. In addition, there is evidence that HMB binds to many structures in the tissue in the form of covalent bonds. These findings suggest that HMB is a component of the cell membrane or other cell structures. Therefore, a logical justification is provided for the function of HMB in protecting muscle tissue. Also, HMB is covalently attached to the forms of many tissues, and it can be

acknowledged that HMB is part of the cell membrane or cell structure. Therefore, the role of HMB in maintaining muscle structure and tissue can be significant (Davies & Kadir, 2012).

The contradiction that exists between the results of this research and the findings of other studies can be partially attributed to the different subjects and sample size, different methods of laboratory measurement, the level of preparation of the subjects and the type of training protocol, limiting the dependent variables, short time. Research and HMB supplement consumption ratio. Also, considering that no significant difference was observed in CK and LDH enzyme indices between the groups taking the HMB supplement and placebo with a dose of 3 grams per day for six days, maybe the dose of 3 grams per day cannot be considered as an inhibitor of high-intensity muscle damage.

What should be considered as the achievement of this research is that the consumption of HMB supplement compared to the training protocol and the people participating in this research could not effectively reduce muscle damage indicators (CK, LDH). Also, liver indices (AST, ALT) not only did not decrease but are known as a factor in aggravating liver damage. It is advised that this research be carried out in various dosages, in various age groups and sexes, in various training protocols, and in both short and long periods of supplement consumption in human samples and animals to be studied to clarify the causes and factors involved in these findings.

### CONCLUSION

The present research results showed that six days of HMB supplementation significantly affected the CK index at different times of the study. Still, no significant changes were observed in the HMB and placebo groups. In addition, no significant difference was observed in the LDH serum levels between and within the subjects at any of the different times of this research. Also, after taking the HMB supplement and placebo for six days at different research times, no significant changes were observed in AST serum levels within and between groups. However, serum ALT levels within the group at different times and between the HMB supplement and placebo groups were significant immediately after the exercise protocol. However, no significant changes were observed in the phase before taking the supplement and placebo and one and 24 hours after the exercise protocol.

### AUTHOR CONTRIBUTION STATEMENT

MJA and SMO were responsible for recruiting volunteers for this experiment and were also involved in data collection procedures; SSM and DLS were responsible for preparing the final manuscript and were involved in the data collection procedures. A manuscript was prepared by HHA and RAA. BJR performed the experimental design and supervised the conduct of the research, and HAM performed the data analysis.

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