

## Research Article

# Influence of B-Hydroxy-B-Methyl Butyrate Supplementation on Strength, Muscle and Liver-Damage Indices Induced by Dual Pyramid Resistance Training in Beginner Bodybuilders

Soleyman Ansari<sup>1</sup>, Shahram Gholamrezaei<sup>2\*</sup>, Fahimeh AdibSaber<sup>2</sup>, Mohammad Moradnia<sup>3</sup>

1. PhD -Exercise Physiology, Department of Physical Education, Rasht Branch, Islamic Azad University, Rasht, Iran
2. Assistant Professor, Department of Physical Education, Rasht Branch, Islamic Azad University, Rasht, Iran
3. MA in Exercise Physiology, Department of Physical Education, Faculty of Humanities, Islamic Azad University, Rasht Branch, Rasht, Iran

**Received:** 4 July 2022

**Revised:** 19 August 2022

**Accepted:** 30 August 2022

### Keywords:

$\beta$ -Hydroxy- $\beta$ -Methyl butyrate, muscle strength, liver-damage markers, muscle-damage indices, dual pyramid resistance training

### Abstract

**Background:** It has been suggested that ingesting supplements decrease muscle soreness, increase muscle strength, hypertrophy, and health-related indices. This study investigated the influence of 8-week  $\beta$ -Hydroxy- $\beta$ -Methyl butyrate (HMB) supplementation combined with a dual pyramid resistance exercise program on strength, muscle, and liver-damage indices (ALT, AST, CK, and LDH) and body mass index (BMI) in beginner bodybuilders.

**Materials and Methods:** A total of 40 beginner bodybuilders was randomized to an intervention group (resistance training + HMB supplementation) and a control group (resistance training + placebo) for 8 weeks. Blood samples and body measurements were taken at baseline and after the end of the intervention. The subjects were evaluated for BMI, 1 repetition maximum (1RM) bench press and leg press prior to and after the training intervention. In addition, blood samples were obtained before and after 8-week resistance training to evaluate creatine kinase (CK), lactate dehydrogenase (LD), aspartate aminotransferase (AST), and alanine aminotransferase (Johnson et al.) responses.


**Results:** After 8 weeks of HMB ingestion, serum levels of CK ( $p < 0.001$ ), LDH ( $p < 0.014$ ), and ALT ( $p < 0.009$ ) of participants in the experimental group significantly decreased compared to the placebo group. Furthermore, The HMB group showed greater gains compared with the placebo group in 1RM leg and bench press ( $p < 0.001$ ). Regarding BMI and AST serum levels, there were no significant differences between groups.

**Conclusion:** The results of the present study showed that HMB supplementation may attenuate the resistance exercise-induced muscle and liver damage indices and have beneficial effects on muscle strength.

\*Corresponding author: Shahram Gholamrezaei

Address: Department of Physical Education, Rasht Branch, Islamic Azad University, Rasht, Iran

Tell: +989111818380 Email: gholamrezaei@iaurasht.ac.ir

 SH GH: 0000-0003-3105-6819

## 1. Introduction

High-intensity resistance training appears essential for improving physical fitness factors, particularly muscular strength and hypertrophy, and is a common form of activity recommended for various healthy age groups and patients (1, 2). Double pyramid resistance training, a combination of the pyramid and reverse pyramid system, uses the maximum number of muscle fibers because they are under the most possible load (3). Repeatedly exposing resistance training exerts mechanical overloads on the body and can stimulate the tissue to adapt with further overload. Muscle and connective tissue damage may occur due to resistance exercises. As a result, bodybuilders need much time to recovery to be able to increase their training load and improve their performance (4).

Creatine kinase (CK) and lactate dehydrogenase (LDH) are two indirect skeletal muscle damage indices. CK is a dimeric globular protein that buffers ATP and ADP concentrations by catalyzing the exchange of phosphate bonds between phosphocreatine and ADP produced during muscular contraction. LDH is an enzyme that catalyzes the conversion of pyruvate to lactate during anaerobic glycolysis (4). Following long-term and strenuous exercises, serum levels of CK and LDH enzymes can dramatically increase (5, 6).

Meanwhile, it has been observed that resistance training may lead to elevations of aspartate aminotransferase (AST) and alanine aminotransferase (7). AST and ALT enzymes are hugely found in the liver. AST is also abundant in other tissues such as the heart, kidneys, skeletal muscle, and red blood cells; but, ALT concentrations in skeletal muscle are low (8).

In fact, an increase in serum level of AST and ALT indicates the entry of muscle and liver enzymes into the bloodstream (6). In addition to this, ALT activation has a high correlation with body mass index (BMI), while AST has a slight one to this variable (6, 9).

It has been suggested that ingesting supplements decrease muscle soreness, increase muscle strength, hypertrophy, and health-related indices (10-12). Over the past 25 years, one dietary supplement that has drawn specific attention in sport is  $\beta$ -hydroxy- $\beta$ -methyl butyrate (HMB) (10, 13). Beneficial effects of HMB – a metabolite of leucine and 2-ketoisocaproic acid – may be correlated with its anti-catabolic action, influence on the de novo synthesis of cholesterol, increased insulin-like growth factor 1 transcription, the stimulation of the mTOR kinase pathway, or the ubiquitin-proteasome system and caspase activity (10, 14).

There have been several studies to investigate the impacts of the ingestion of HMB in combination with resistance training in terms of improving strength, and damage markers. Asjodi and Izadi (2019), Standley et al. (2017), van Someren et al. (2005) and Panton et al (15) reported that HMB ingestion (3 g/day) during 3–8 weeks of resistance training decreased CK and LDH, lowered inflammatory biomarkers, and improved body composition; However, Arazi et al.(2015), Kirby et al.(2010), Lambole et al (2007), and Shirato et al. (2016) were found no significant differences between HMB and placebo groups regarding blood lipids, muscle damage and body composition.

In general, muscle fibers damage and inflammatory responses due to intense and eccentric exercises reduce athlete's performance in subsequent sessions; so, it is essential to prevent injuries in the athletes for further efforts. To best our knowledge, the long-term effects of the combination of HMB ingestions and resistance training on liver-damage indices have not been reported yet in previous studies. According to the previous contradictory results and the increase in HMB consumption among resistance and competitive athletes, the aim of the present study was to investigate the influence of HMB supplementation with dual pyramid resistance exercises on muscle and liver damage enzymes, muscle strength and BMI in beginner bodybuilders.

## 2. Materials and Methods

This randomized, double-blind, placebo-controlled trial with pre and post-test design approved by the Ethics Committee of Azad University of Rasht (IR.IAU.RASHT.REC.1399). The trial has been registered in the Iranian Registry of Clinical Trials (IRCT) as IRCT20180503039517N4.

### Participants

The study population consisted of 53 beginner males (aged 19–29) bodybuilders who were members of a sports club in Lahijan City, Iran. Inclusion criteria were: 1) a maximum of 3 months involving in resistance exercise, 2) no smoking, 3) no history of cardiorespiratory, musculoskeletal, neurological, hormonal or orthopedic disorders, 4) no change in daily diet during the study, and 5) no ingestion of androgenic and energetic supplements in the past 1 months. Exclusion criteria included 1) absence for more than two sessions through intervention, 2) consumption of additional supplementation during the study, and 3) suddenly being disease.

### Randomization and study groups

The sample comprised 40 amateur bodybuilders who were voluntarily recruited and then randomly divided into two groups: HMB supplementation+ resistance training (n=20) and placebo+ resistance training (n=20). A priori calculations of statistical power using G \* Power indicated that this sample size was appropriate to satisfy power at or above 80% (16). A researcher not involved with data collection was responsible for randomization and group allocation (using a computer-generated randomization sequence). Participants signed informed consent and were completely voluntary to withdraw at any stage of research. They were screened by a physician to be eligible to participate in exercise training. None of them received any muscle-building supplements before.

All subjects completed a familiarization session with equipment, resistance training room, and proper strength tests one week prior to the initiation of the resistance training intervention.

For HMB supplementation group, HMB capsules containing 1 g of HMB per capsule were taken (Optimum Nutrition, Inc., USA): One capsule was ingested 3 times a day before breakfast, lunch, and supper (HMB: 3 g/day) on non-training days. Moreover, on training days, participants took three capsules of the assigned preparation per day in three doses as follows: before breakfast and supper, and 30 minutes before training, as well. Each serving of placebo contained the same amount of polydextrose (17). It should be reminded that both HMB and placebo capsules were the same in size and appearance.

Resistance training involved an eight-week dual pyramid pattern program (3 sessions per week). Each session consisted of a 10 minutes warm-up, 65 minutes of main exercises, and 5 minutes cool down. The main part of training program included the 8 exercises in each session (8 sets with a break of 5 minutes in the first 2 weeks, 4 minutes in the third and fourth weeks and 3 minutes in the last 4 weeks) (18) (resistance program was shown in table 1 in detail). They were closely supervised by a trained exercise specialist to assure performing techniques properly. During this pretest and posttest scheduling food intake was controlled by an investigator by means of an interview. All participants completed training sessions in full.

**Table 1:** Main dual pyramid resistance training program

Program activity	duration	Content	
Warm up	10 min	jogging and stretching (focusing on the muscle groups that were about to be trained)	
Main dual pyramid resistance training program	65 min	movements	Set = reps × load
		bench press, leg press, hamstring curl, knee extension, cable biceps curl and triceps extension	1= 4 × 80% 1RM
			2= 3 × 85% 1RM
			3= 2 × 90% 1RM
			4= 1 × 95% 1RM
			5= 1 × 95% 1RM
			6= 2 × 90% 1RM
			7= 3 × 85% 1RM
8= 4 × 80% 1RM			
Cool down	5 min	The same exercises as warm up	

## Measures

### Baseline anthropometric and physical fitness assessment

Forty-eight hours before and after the intervention, height, weight, BMI were measured by dividing body weight (kg) by the square of the height (m<sup>2</sup>) of the subject. For the upper and lower body strength, bench and leg press exercises were measured by free weights (Mobarez Company, Tehran, Iran) as mentioned before (19). One RM was assessed in knee extension, hamstring curl, leg press, bench press, cable biceps curl, and triceps pushdown exercises. Briefly, the participants performed a warm-up which consisted of jogging, static and dynamic stretching and exercises. Two to three trials separated by 2-3 minutes of rest were used to determine the individuals' 1RM for each resistance exercise. In these sessions, a weight that could be lifted maximally to fatigue after 2-10 repetitions was used to calculate 1RM according to the formula proposed by Brzycki (20).

### Laboratory investigations

All laboratory measurements were performed one day before and two days after the intervention period. Trained and experienced phlebotomists collected ten-milliliter venous blood samples in a seated position after 12 -h overnight fasting between 7:30–9 a.m. The serum was separated and stored at -80°C until analysis. In both stages, the serum level of CK, LDH, ALT, and AST enzymes were measured by an enzymatic colorimetric method (Pars Azmoun, Tehran, Iran).

## Statistical Analyses

The normality of all distributions was determined by Shapiro-Wilk test, a paired sample t-test was used to calculate the statistical significance of the difference between pre and post measurements within each group. Independent t-test was also used to compare the differences in change scores (post-test minus pre-test scores for each subject) between intervention and control groups. To calculate an effect size Cohen's d was used with the parameter;  $d \leq 0.2$  considered small,  $> 0.8$  large, and between these values moderate (21). Standard statistical procedures were selected for the calculation of means, standard deviations and 95% confidence intervals. Statistical significance was set at  $p < 0.05$ . All analyses were conducted using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA).

### 3. Results

In total, 40 beginner bodybuilders (mean age  $22.85 \pm 2.98$  years) were recruited for this study. In order to examine the differences between the two groups in baseline scores, we performed an independent t-test. At baseline, no significant difference was found between the intervention and control groups regarding the participants' demographic features (age, height, weight, and BMI) and research variables (leg press, bench press, BMI, CPK, LDH, ALT and AST values) ( $p > 0.05$ ) (see Table 2).

**Table 2:** Baseline Measurements

	HMB (n=20) Mean $\pm$ SD	Placebo (n=20) Mean $\pm$ SD	p-value*
Baseline demographics			
Age (year)	22.45 $\pm$ 2.91	23.45 $\pm$ 3.15	0.140
Height (m)	1.74 $\pm$ 0.07	1.71 $\pm$ 0.074	0.834
Wight (kg)	73.9 $\pm$ 9.7	70.6 $\pm$ 7.67	0.240
BMI (kg/m <sup>2</sup> )	25.18 $\pm$ 2.1	24.03 $\pm$ 2.52	0.126

\*p Values were derived using two-tailed, independent-samples t-tests.

In order to investigate the effect of dual pyramid resistance training combined with HMB or placebo on research variables, the independent t-test was conducted to compare mean changes ( $\Delta$ ) of the variable values between the HMB and placebo groups (See table 3).

**Table 3:** Comparison of research variable scores and mean changes ( $\Delta$ ) of the aquatic and control groups.

Variable s	HMB group (n=20)			Placebo group (n=20)			ES [%95CI]	t	p
	Pre Mean (SD)	Post Mean (SD)	$\Delta$ Mean (SD)	Pre Mean (SD)	Post Mean (SD)	$\Delta$ Mean (SD)			
<b>BMI</b> (kg/m <sup>2</sup> )	25.18 (2.1)	24.92 (2.07)	0.25 (0.83)	24.03 (2.52)	24.15 (2.24)	0.11 (1.08)	0.14 [-0.24,0.99]	1.22	0.228
<b>Leg Press</b>	158.6 (6.23)	173.50 (9.31)	-14.9 (6.73)	158.95 (7.65)	163.00 (7.55)	4.05 (0.75)	2.26 [-13.91, - 7.78]	-7.15	0.001
<b>Bench Press</b>	53.25 (5.39)	61.6 (5.73)	-8.35 (4.09)	51.00 (5.74)	55.75 (5.63)	4.75 (0.96)	0.141 [-5.55, -1.64]	3.82	0.001
<b>CPK</b> (U/L)	256.1 (89.26)	190.45 (76.15 )	65.65 (52.58 )	253.1 (72.3)	244.95 (72.60 )	8.15 (4.30)	1.54 [33.61, - 81.38]	4.87	0.001
<b>LDH</b> (U/L)	349.05 (64.07)	317.9 (61.21 )	31.15 (35.1)	331.8 (67.6)	321.95 (68.08 )	9.85 (3.95)	0.85 [5.29, - 37.30]	2.69	0.014
<b>ALT</b> (mg/dl)	25.80 (9.44)	19.20 (4.33)	6.60 (8.81)	26.95 (8.59)	26.10 (8.44)	0.85 (1.18)	0.91 [1.72, -9.77]	2.89	0.009
<b>AST</b> (mg/dl)	22.85 (4.72)	18.00 (3.78)	4.85 (4.25)	21.85 (5.54)	17.90 (3.83)	0.11 (0.74)	1.55 [-1.65,3.45]	0.713	0.48



Paired samples t-tests were used to compare within groups changes. After 8 weeks exercise, the HMB group showed statistically increase for bench press 1RM record (improvement =8.35, effect size = 0.151, 95% CI [6.43, 10.26],  $t(19) = 9.12$ ,  $p < .001$ ), and the leg press record (improvement =14.90, effect size = 0.093, 95% CI [11.74, 18.05],  $t(19) = 9.89$ ,  $p < .001$ ). Moreover, the HMB group showed significant decrease for CK (improvement = -65.60, effect size = 0.25, 95% CI [-90.26, -41.03],  $t(19) = -5.58$ ,  $p < .001$ ), LDH (improvement = -65.60, ES = 0.089, 95% CI [-47.59, -14.70],  $t(19) = -3.96$ ,  $p < .001$ ), ALT (improvement = -6.60, effect size = 0.25, 95% CI [-10.72, -2.47],  $t(19) = -3.35$ ,  $p < .003$ ), and AST values (improvement = -4.85, effect size = 0.20, 95% CI [-6.84, -2.85],  $t(19) = -5.09$ ,  $p < .001$ ).

On the other hand, After 8 weeks exercise, The placebo group showed statistically increase for bench press record (improvement =4.75, effect size = 0.09, 95% CI [4.29, 5.20],  $t(19) = 21.97$ ,  $p < .001$ ), and leg press record (improvement =0.025, effect size = 0.093, 95% CI [3.69, 4.40],  $t(19) = 23.85$ ,  $p < .001$ ). Moreover, the placebo group showed significant decreases for CK (improvement = -8.15, effect size = 0.032, 95% CI [-10.16, -6.13],  $t(19) = -8.46$ ,  $p < .001$ ), LDH (improvement = -8.15, ES = 0.029, 95% CI [-11.69, -8.00],  $t(19) = -11.15$ ,  $p < .001$ ), ALT (improvement = -0.85, effect size = 0.031, 95% CI [-1.40, -0.29],  $t(19) = -3.21$ ,  $p < .005$ ), and AST values (improvement = -3.95, effect size = 0.180, 95% CI [-5.68, -2.21],  $t(19) = -4.76$ ,  $p < .001$ ). BMI was compared in both groups before and after intervention. The results indicated no significant change in BMI in HMB ( $p > 0.189$ ) and placebo ( $p > 0.628$ ) groups.

## 4. Discussion

The present study aimed to examine the effect of HMB supplementation on liver and muscle damage markers, BMI, and muscle strength after 8 weeks of dual pyramid resistance training in amateur bodybuilders. Our results demonstrated that there was a significant difference in CK and LDH serum levels, between two groups, following 8 weeks of resistance training. In relation to the pre-investigation value, after HMB supplementation and placebo, the changes in CK and LDH were significant; however, the HMB supplementation group indicated greater meaningful changes.

Our results are consistent with those of some researchers (10, 22-24). Asjodi et al investigated the effects of 8 weeks HMB supplementation (3 g/day) on body composition, CK, and LDH following exercise. Results revealed that the values of CK and LDH in the HMB group were significantly lower compared to the placebo group. Durkalek-Michaleski and Jeszka, also reported that HMB ingestion (3 g/day) during 12 weeks of resistance training decreased CK and LDH activity. Furthermore, Nissen et al. and van Someren et al., following HMB supplementation, found a lower activity of CK and/or LDH in the blood of examined individuals.

In contrast, there is evidence of no change in the serum levels of muscle damage parameters following HMB supplementation. Shirato et al and Wilson et al suggested that the ingestion of HMB does not have a role to reduce muscle damage markers after resistance exercise. The discrepancy between this study and previous research may be attributed to the length of supplementation prior to the eccentric exercise. In this study, subjects ingested the HMB supplement 30 minutes before the exercise bout.



However, the duration to take the supplement was 60 minutes prior to the eccentric exercise trial in Wilson's study. In addition, it may be due to various supplementary periods in two studies (7 days before and 4 days after one special eccentric exercise in Shirato's study versus 24 sessions in 8-week supplementation (3 meals per day) in our study.

The other findings of our study revealed that there was a significant difference between the two groups in the serum level of ALT, after 8 weeks of resistance training, but we could not observe a significant difference in AST serum level between the groups. In relation to the pre-investigation value, after HMB supplementation and placebo, the changes in ALT and AST were significant.

To our knowledge, this is the first study to investigate the effects of long-term HMB supplementation on serum levels of liver enzymes following 8 weeks of resistance exercise. Only one study examined the effects of short-term HMB supplementation on serum AST and ALT after intense resistance exercise in untrained male students (25). Contrary to our results, Saki et al concluded that consumption of HMB before exercise did not have significant effects on serum AST and ALT. It should be noted that different lengths of supplementation (6 days before weight training) and shorter approach of the intervention (a single session of exercise) could be the possible reason for the contradiction between the results of the present study and the mentioned study.

It has been shown that intense resistance training resulted in profound increases in the muscle and liver function parameters, which may cause increased levels of CK and LDH, as well as in AST and ALT levels (8, 25). The beneficial effects of HMB combined with resistance training have been reported in several studies. It has been demonstrated that the utilization of HMB has the ability to debilitate muscle damage indices and improve recovery (2, 22, 26). One explanation for the positive results of HMB supplementation relates to the fact that HMB is a precursor of cholesterol amalgamation in skeletal muscle. This has prompted the theory that HMB would raise post-training cholesterol synthesis, along these lines quickening the repair of training-induced muscle damage. This, in turn, would permit for increased training loads finally leading to elevated hypertrophic responses (27).

Finally, the present results addressed that there was a significant difference in muscle strength values (1RM leg press and bench press), between two groups, after 8 weeks of resistance training. In relation to the pre-investigation value, after HMB supplementation and placebo, the changes in upper and lower extremity muscle strength were significant; however, the HMB supplementation group indicated greater meaningful changes. Moreover, no significant differences were observed in BMI between HMB and placebo groups, after resistance training.

Lamboley et al's result (28) is not agreeing with our results. They could not observe a significant effect of HMB on BMI in the active college students. To explain the difference between the findings, it should be noted that the type of training program (aerobic program) and the supplementation period (5 weeks) were different.

On the other hand, Asjodi et al, Asadi et al and Panton et al reported that HMB supplementation significantly improved muscle strength and BMI, which are in line with our findings. Two mechanisms for HMB action have been proposed. The first depends on the perception that HMB can slow or restrain muscle proteolysis that is increased during exercise. It can also improve fatty acid oxidation and cause increases in fat-free mass, and enhances the biochemical mechanisms necessary for protein synthesis (22, 29). The second probable mechanism concerns HMB being an antecedent of muscle-cell cholesterol. The expansion in muscle hypertrophy during weight training may bring about a local shortage in cholesterol in the muscle cell, which might result in insufficient cholesterol for membrane synthesis and consequently slower cell growth or sub-optimally functioning cell membranes. Providing basic measures of cholesterol precursors in muscle would permit the muscle to maintain and synthesize new muscle plasma membranes (30). The present study has certain limitations. First, the study included only beginner bodybuilders; so, the results might not be generalizable to intermediate or elite athletes. Second, the population in this study were young (19 to 29 years), which may not be suitable for popularizing the results to other age groups.

## ∆. Conclusion

According to the findings of the present study, it can be concluded that 8-week ingestion of HMB may have a positive effect on muscle damage markers after the dual pyramid resistance exercise of amateur bodybuilders. Additionally, HMB consumption combined with resistance exercises could induce meaningful increases in muscle strength.

## Acknowledgements

The authors would like to thank all athletes who participated in this research study. In addition, we thank the laboratory staff.

## Funding

This study did not have any funds.

## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest in publishing this article.

**Ethical approval** This article was derived from a master's thesis on Exercise Physiology with the project code: 981480 and the ethical code: IR.IAU.RASHT.REC.1399, Ethics Committee of Azad University of Rasht, Rasht, Iran. This study was also registered in the Iranian Registry of Clinical Trials with the code: IRCT20180503039517N4.

**Informed consent** Informed consent was obtained from all participants

## Author contributions

Conceptualization: S.A., M.M.; Methodology: M.M., F.A.; Software: SH.GH.; Validation: S.A., M.M.; Formal analysis: F.A., SH.GH.; Investigation: S.A., F.A.; Resources: S.A., M.M., F.A.; Data curation: M.M., SH.GH.; Writing - original draft: S.A., M.M.; Writing - review & editing: F.A., S.A.; Visualization: M.M., SH.GH.; Supervision: S.A.; Project administration: M.M.; Funding acquisition: SH.GH., F.A.

## References

1. Fleck SJ, Kraemer W. Designing resistance training programs, 4E: Human Kinetics; 2014.
2. Shirato, M., Tsuchiya, Y., Sato, T. *et al.* Effects of combined  $\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB) and whey protein ingestion on symptoms of eccentric exercise-induced muscle damage. *J Int Soc Sports Nutr* **13**, 7 (2016). <https://doi.org/10.1186/s12970-016-0119-x>
3. Bompa T, Buzzichelli C. Periodization training for sports, 3e: Human kinetics; 2015.
4. Kirby TJ, Triplett NT, Haines TL, Skinner JW, Fairbrother KR, McBride JM. Effect of leucine supplementation on indices of muscle damage following drop jumps and resistance exercise. *Amino Acids*. 2012 May;42(5):1987-96. doi: 10.1007/s00726-011-0928-9. Epub 2011 May 12. PMID: 21562819.
5. Cooke MB, Rybalka E, Williams AD, Cribb PJ, Hayes A. Creatine supplementation enhances muscle force recovery after eccentrically-induced muscle damage in healthy individuals. *J Int Soc Sports Nutr*. 2009 Jun 2;6:13. doi: 10.1186/1550-2783-6-13. PMID: 19490606; PMCID: PMC2697134.
6. R Pincus M, A McPherson R. Henry's Clinical Diagnosis and Management by Laboratory Methods, 23e. Elsevier; 2017.
7. Johnson NA, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson MW, George J. Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology*. 2009 Oct;50(4):1105-12. doi: 10.1002/hep.23129. PMID: 19637289.
8. Pettersson J, Hindorf U, Persson P, Bengtsson T, Malmqvist U, Werkström V, Ekelund M. Muscular exercise can cause highly pathological liver function tests in healthy men. *Br J Clin Pharmacol*. 2008 Feb;65(2):253-9. doi: 10.1111/j.1365-2125.2007.03001.x. Epub 2007 Aug 31. PMID: 17764474; PMCID: PMC2291230.
9. Brancaccio P, Lippi G, Maffulli N. Biochemical markers of muscular damage. *Clin Chem Lab Med*. 2010 Jun;48(6):757-67. doi: 10.1515/CCLM.2010.179. PMID: 20518645.
10. Durkalec-Michalski K, Jeszka J. The efficacy of a  $\beta$ -hydroxy- $\beta$ -methylbutyrate supplementation on physical capacity, body composition and biochemical markers in elite rowers: a randomised, double-blind, placebo-controlled crossover study. *J Int Soc Sports Nutr*. 2015 Jul 30;12:31. doi: 10.1186/s12970-015-0092-9. PMID: 26225130; PMCID: PMC4518594.
11. Wilson JM, Kim JS, Lee SR, Rathmacher JA, Dalmau B, Kingsley JD, Koch H, Manninen AH, Saadat R, Panton LB. Acute and timing effects of beta-hydroxy-beta-methylbutyrate (HMB) on indirect markers of skeletal muscle damage. *Nutr Metab (Lond)*. 2009 Feb 4;6:6. doi: 10.1186/1743-7075-6-6. PMID: 19193206; PMCID: PMC2642830.
12. Wilson JM, Lowery RP, Joy JM, Andersen JC, Wilson SM, Stout JR, Duncan N, Fuller JC, Baier SM, Naimo MA, Rathmacher J. The effects of 12 weeks of beta-hydroxy-beta-methylbutyrate free acid supplementation on muscle mass, strength, and power in resistance-trained individuals: a randomized, double-blind, placebo-controlled study. *Eur J Appl Physiol*. 2014 Jun;114(6):1217-27. doi: 10.1007/s00421-014-2854-5. Epub 2014 Mar 6. PMID: 24599749; PMCID: PMC4019830..
13. Nunes EA, Lomax AR, Noakes PS, Miles EA, Fernandes LC, Calder PC.  $\beta$ -Hydroxy- $\beta$ -methylbutyrate modifies human peripheral blood mononuclear cell proliferation and cytokine production in vitro. *Nutrition*. 2011 Jan;27(1):92-99. doi: 10.1016/j.nut.2009.12.008. Epub 2010 Jun 11. PMID: 20541366.
14. Ellis AC, Hunter GR, Goss AM, Gower BA. Oral Supplementation with Beta-Hydroxy-Beta-Methylbutyrate, Arginine, and Glutamine Improves Lean Body Mass in Healthy Older Adults. *J Diet Suppl*. 2019;16(3):281-293. doi: 10.1080/19390211.2018.1454568. Epub 2018 Apr 19. PMID: 29672184; PMCID: PMC6314919..
15. Panton LB, Rathmacher JA, Baier S, Nissen S. Nutritional supplementation of the leucine metabolite beta-hydroxy-beta-methylbutyrate (hmb) during resistance training. *Nutrition*. 2000 Sep;16(9):734-9. doi: 10.1016/s0899-9007(00)00376-2. PMID: 10978853.

16. Faul F, Erdfelder E, Lang AG, Buchner A. G\*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007 May;39(2):175-91. doi: 10.3758/bf03193146. PMID: 17695343.
17. Asadi A, Arazi H, Suzuki K. Effects of  $\beta$ -Hydroxy- $\beta$ -methylbutyrate-free Acid Supplementation on Strength, Power and Hormonal Adaptations Following Resistance Training. *Nutrients*. 2017 Dec 2;9(12):1316. doi: 10.3390/nu9121316. PMID: 29207472; PMCID: PMC5748766.
18. Bompa T, Di Pasquale M, Cornacchia L. *Serious strength training: Human Kinetics*; 2012.
19. *Medicine ACoS. ACSM's resource manual for guidelines for exercise testing and prescription: Lippincott Williams & Wilkins*; 2012.
20. ARAZI, H., & ROHANI, H., & GHIASI, A., & DAVARAN, M. (2015). THE EFFECT OF HMB SUPPLEMENTATION ON CARDIOVASCULAR RISK FACTORS AFTER FOUR WEEKS OF RESISTANCE TRAINING IN AMATEUR ATHLETES. *INTERNATIONAL CARDIOVASCULAR RESEARCH JOURNAL*, 9(2), 89-93. <https://www.sid.ir/en/journal/ViewPaper.aspx?id=436571>
21. Cohen J. *Statistical power analysis for the behavioral sciences: Academic press*; 2013.
22. Asjodi F, Izadi A. The Effects of 8 weeks beta-hydroxy-beta-methylbutyrate (HMB) supplementation on body composition, inflammatory response and muscle damage after eccentric exercise in untrained males. *Progress In Nutrition*. 2019;21:184-90. <https://doi.org/10.23751/pn.v21i1-S.5883>
23. Nissen S, Sharp R, Ray M, Rathmacher JA, Rice D, Fuller JC Jr, Connelly AS, Abumrad N. Effect of leucine metabolite beta-hydroxy-beta-methylbutyrate on muscle metabolism during resistance-exercise training. *J Appl Physiol* (1985). 1996 Nov;81(5):2095-104. doi: 10.1152/jappl.1996.81.5.2095. PMID: 8941534.
24. van Someren KA, Edwards AJ, Howatson G. Supplementation with beta-hydroxy-beta-methylbutyrate (HMB) and alpha-ketoisocaproic acid (KIC) reduces signs and symptoms of exercise-induced muscle damage in man. *Int J Sport Nutr Exerc Metab*. 2005 Aug;15(4):413-24. doi: 10.1123/ijsnem.15.4.413. PMID: 16286672.
25. Saki B, Gaeini AA, Choubineh S. The Effects of Short-Term  $\beta$ -Hydroxy- $\beta$ -Methylbutyrate Supplementation on Serum AST, ALT, AP, and Urea Levels after Intense Resistance Exercise in Untrained Male Students. *Journal of Isfahan Medical School*. 2012;30(190).
26. Nissen S, Sharp RL, Panton L, Vukovich M, Trappe S, Fuller JC Jr. beta-hydroxy-beta-methylbutyrate (HMB) supplementation in humans is safe and may decrease cardiovascular risk factors. *J Nutr*. 2000 Aug;130(8):1937-45. doi: 10.1093/jn/130.8.1937. PMID: 10917905..
27. Tritto AC, Bueno S, Rodrigues RMP, Gualano B, Roschel H, Artioli GG. Negligible Effects of  $\beta$ -Hydroxy- $\beta$ -Methylbutyrate Free Acid and Calcium Salt on Strength and Hypertrophic Responses to Resistance Training: A Randomized, Placebo-Controlled Study. *Int J Sport Nutr Exerc Metab*. 2019 Sep 1;29(5):505-511. doi: 10.1123/ijsnem.2018-0337. PMID: 30859862..
28. Lamboley CR, Royer D, Dionne IJ. Effects of beta-hydroxy-beta-methylbutyrate on aerobic-performance components and body composition in college students. *Int J Sport Nutr Exerc Metab*. 2007 Feb;17(1):56-69. doi: 10.1123/ijsnem.17.1.56. PMID: 17469236..
29. Flakoll P, Sharp R, Baier S, Levenhagen D, Carr C, Nissen S. Effect of beta-hydroxy-beta-methylbutyrate, arginine, and lysine supplementation on strength, functionality, body composition, and protein metabolism in elderly women. *Nutrition*. 2004 May;20(5):445-51. doi: 10.1016/j.nut.2004.01.009. PMID: 15105032..
30. Jówko E, Ostaszewski P, Jank M, Sacharuk J, Zieniewicz A, Wilczak J, Nissen S. Creatine and beta-hydroxy-beta-methylbutyrate (HMB) additively increase lean body mass and muscle strength during a weight-training program. *Nutrition*. 2001 Jul-Aug;17(7-8):558-66. doi: 10.1016/s0899-9007(01)00540-8. PMID: 11448573..