JCHR (2023) 13(3), 547-555

Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Comparison of Six Weeks of Weight Training with a Combination of Beta-hydroxy-Beta-methylbutyrate and Creatinine and Two Weeks of Non-training on the Concentrations of some Blood Biochemical Factors in Male Powerlifting Athletes

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(Received: 22 April 2021 Accepted: 13 September 2021)

KEYWORDS

Creatinine supplement; Beta-hydroxymethyl Butyrate; High-Density Lipoprotein; Low-Density Lipoprotein; Cholesterol; Triglyceride

ABSTRACT: In this study, concentrations of some blood biochemical factors in male powerlifting athletes were compared through 6 weeks of weight training with combined beta-hydroxy-beta-methylbutyrate (HMB) and creatinine (Cr) supplements and 2 weeks of non-training. Thirty-two male athletes aged 21-30 years were randomly divided into four groups of eight individuals: Cr, HMB, HMB + Cr, and weight training. The training program was designed for 6 weeks and four sessions per week. The HMB group consumed 3 g of the supplement on training days and 1g on non-training days, the Cr group consumed three servings of 5 g on training days and 5 g on non-training days, and the HMB +Cr group consumed the same amount of HMB and Cr supplements as the HMB and Cr groups. During 2 weeks of non-training, the HMB, Cr, and HMB + Cr groups received daily 1 g, 5 g, and 1 g of HMB + 5 g of Cr, respectively. The hypotheses were tested by two-way repeated-measures analysis of variance (ANOVA). Results: There were no significant differences in Cholesterol (Chol.), Triglyceride (TG), and Low-Density Lipoprotein (LDL) levels after the post-test and 2 weeks of non-training. There were significant differences in High-Density Lipoprotein (HDL) in post-test between groups of weight training and HMB ($p = 0.000$), weight training and HMB + Cr ($p = 0.002$), Cr and HMB ($p = 0.000$), and Cr and HMB + Cr ($P = 0.001$). The weight training and HMB ($p = 0.000$), weight training and HMB + Cr ($p = 0.003$), Cr and HMB (p 0.000) =), and Cr and HMB + Cr groups ($p = 0.001$) were significantly different in 2 weeks of non-training. Conclusion: Six-week weight training with combined HMB and Cr supplement and 2 weeks of non-training can significantly change the concentrations of some blood biochemical factors in male powerlifting athletes.

INTRODUCTION

Research evidence has shown that overweight and obesity are associated with elevated serum lipids and lipoproteins, which in turn increase serum lipids as body fat increases [1]. Weight loss programs can possibly affect serum cholesterol, triglycerides, and lipoproteins [2], hence, it can be hoped that some physical biochemical factors [Triglyceride (TG), Cholesterol (Chol), Low-density lipoprotein (LDL), and high-density lipoprotein (HDL)] in the blood can be reduced through physical activity and the use of some permitted supplements [3].

Cholesterol is a major component of cell membranes and is essential not only for cell wall integrity but also for the regulation of intracellular processes [4]. It is a

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DOI: 10.22034/jchr.2021.1928596.1297

soft, fat-like substance that is found in the bloodstream and all the cells of the body and is divided into two types: LDL (bad cholesterol) and HDL (good cholesterol). The function of LDL in the body is to transport cholesterol and other types of fats in the blood. If the blood LDL level is higher than normal, it can narrow the arteries and damage the coronary arteries, causing serious problems for the brain and heart. High LDL increases the risk of heart attack, cardiac arrest, and stroke. On the other hand, HDL is considered good cholesterol. Therefore, high LDL and low HDL are risk factors for atherosclerosis while a low LDL to HDL ratio is very beneficial for the body. The rapid removal and reduction of LDL cholesterol from the blood are associated with the number of active receptors on the surface of liver cells [5]. Triglycerides are the most abundant fats in the human body that are stored as fat in the body and more than 99% of body fat is in the form of triglycerides [6]. Findings show that the program of resistance training of trunk muscles significantly reduces cholesterol and triglycerides [3]. Therefore, exercise not only reduces total blood cholesterol but also increases the part of cholesterol, called HDL_C lipoprotein, and reduces the other part, which is LDL-C lipoprotein [7]. Increasing physical activity reduces the amounts of total cholesterol, triglycerides, and LDL and increases the amount of HDL [8]. Many athletes use licensed dietary supplements to improve the quality and quantity and maintain the performance of their workouts in a competitive and training environment. By taking the right nutritional supplements at the right time and in the right way, they can maximize their athletic performance and increase their performance. For this purpose, supplements are recommended to strengthen the performance of athletes. Examples of such a supplement are beta-hydroxy beta-methyl butyrate (HMB) and creatinine (Cr). HMB supplement is the essential amino acid metabolite of leucine. In our body, HMB is supplied by protein consumed by the diet, and HMB supplements reduce the process of proteolysis, which is a natural process of muscle tissue breakdown that occurs after strenuous physical activity such as exercise. Using HMB helps the body accelerate the regeneration process by reducing muscle breakdown and reducing protein after exercise. This means that the body stays in an anabolic state for a longer period of time, which leads to increased muscle growth [9].

HMB supplement did not significantly lower LDL-C in subjects with acceptable normal cholesterol levels (less than 200 mg/dl) [10]. This suggests that HMB is effective in lowering LDL-C when cholesterol levels are high. Cholesterol is produced from acetyl COA, which is synthesized in a slowing step from Mualonic acid, which is the precursor of cholesterol in the cytosol of muscle and liver cells. This process is performed by the enzyme hydroxy–methyl-glutaryl-reductase. Most of HMB is converted to HMB-CoA and then to cholesterol [11]. Damaged muscle lacks the ability to produce the amount of cholesterol needed to stabilize sarcoma. Consumption of HMB can be effective in regenerating and repairing cell walls that have been damaged during intense physical function [3].

Another supplement is Cr supplement. Cr is a natural element in the diet that is synthesized in the body by the liver. Cr supplementation loads the muscle with Cr and increases its total stores in free and phosphorylated forms (so-called creatinine and phosphocreatinine, PCRN). This substance is formed in the body as a phosphate compound (creatinine phosphate) and is used as a source of energy storage, especially in activities and sports of speed and explosions. Most of Cr is stored in skeletal muscles. Cr has now been shown to play a major role in the phosphagen apparatus (ATP-PC), and 95% of the body's Cr is stored in the muscular system, with the rest found mainly in the heart, brain, and testicles [12]. Two-thirds of the stored form of Cr is stored as PCRN and the remaining in its original form. The body needs an average of 2 g of Cr per day depending on the body weight and the activity level [13]. The effects of long-term use (more than 1 week) of the Cr supplement on the performance of athletes have been studied in various sports, especially athletes in speed and strength [14]. The most important function of Cr is to support the regeneration of ATP in the phosphagen energy system. Cr supplementation increases PCRN, free Cr, and total muscle Cr [15].

Weight training is a common type of strength (resistance) training in which weights are used to increase the strength and size of skeletal muscles. Regarding the effect of resistance activities on cardiovascular risk factors, researchers have shown that resistance training reduces LDL and increases HDL in the blood [16]. Strength and resistance training also have protective benefits, and doing such exercises can improve or maintain the lipid status of blood [17]. The potential role of resistance training in improving physical factors has also been demonstrated elsewhere [18].

Many studies have investigated the effect of each of these supplements alone on the biochemical factors of athletes' blood, but the combined effect of these two supplements has been less studied previously, and limited relevant studies are available. Therefore, this study aimed to examine the effect of 6 weeks of weight training with a combination of HMB and the Cr supplement and 2 weeks of non-training on the concentrations of some blood biochemical factors (Chol., TG, LDL, and HDL) in male powerlifting athletes.

MATERIALS AND METHODS

The statistical population of this study included 100 male powerlifting athletes in Semnan city. Among them, 32 statistical samples were selected with an age range of 21-30 years, who had at least 3 years of experience working with weights. The subjects presented consent forms to participate and cooperate in the research and then completed the medical-sports questionnaire. Subjects were then matched based on height (mean height 175.62 ± 7.93), weight (mean weight 74.17 ± 9.96), and age in four groups. They were randomly divided into four groups supplemented with Cr, HMB, HMB + CR, and weight training (without supplementation).

Instruments

At first, the subjects were explained about the research method and a consent form was signed by them to participate in the research. One week before the main test, the subjects' heights and weights were measured, and they were matched in terms of age, height, and weight. The height of the subjects was measured using a SECA ---gauge device (Germany) with an accuracy of 0.1 cm, and their weight was measured using a SECA digital scale with an accuracy of 0.01 kg.

Procedure

The training program (Table 1) of the four groups was designed for 6 weeks and four sessions per week.

Table 1. Weight training program over 6 weeks

	1) Exercise with stretching movements for 5-10 min
	2) Chest press with barbell: 2 sets of 15 to warm up the muscles and 5 sets of 5
	3) High-chest presses with harebell: 4 sets of 6
SAT	4) Chest on a flat table: 6-8-10
	5) Parallel preferably with weight: 2 sets of 15
	6) The back of the wire harness: 8-8-10-12
	7) Laying on the back of the barbell arm with the bar bent: 6-8-10-12
	8) The back of the chest press arm: 5 sets of 5
	1) Exercise with stretching movements 5-10 min
	2) The front of the machine: 10-10-12-15
	3) Squat with harebell: 2 sets of 15 to warm up the muscles and 5 sets of 5
	4) Machine foot press 6-8-10-12
	5) Lift the back of the leg with a barbell: 6-8-10-12
SUN	6) Waist fillet or Japanese greeting: 2 sets of 15
	7) Publish by sitting dumbbells: 8-10-12-12
	8) The barbell shoulder sits from behind: 6-8-10-10
	9) Publishing from the front with a barbell: 3 sets of 12
	10) Head barbell lift: 8-8-8
	11) The abdomen with 90◦ knees: 3 sets of 25
TUE	1) Exercise with stretching movement 5-10 min

	2) Open-handed horizontal bar: maximum two sets 3) Deadline: 5 sets of 5 4) The armpit of the wire is gathered from the front of the hand in revers: 4 sets of 10 5) Armpit boat: 4 sets of 8 6) Forearm, barbell standing, hand-folded: 6-8-10-12 7) The dumbbell is bent in front of the arm: 8-8-10-12 8) Larry dumbbell front arm: 3 sets of 12 9) Forearm: 2 sets of 25
WED	1) Exercise with stretching movements 5-10 min 2) The front of the machine: 4 sets of 12 3) Scott barbell from the front: 4 sets of 8 4) The back of the machine: 4 sets of 10 5) Chest press with barbell: 3 sets of 8 6) High dumbbell chest press: 3 sets of 12 7) Cross over: 2 sets of 12 8) Behind the arm wire hand in reverse: 3 sets of 12 9) Behind the single dumbbell arm, behind the neck two-hand sitting: 3 sets of 10 10) Sitting leg: 3 sets of 15

Before the start of each training session, the subjects warmed up for 5-10 min using stretching movements. Using the adjusted training program (Table 1), they then performed the exercises using a bodybuilding machine (Sayyas Peikar model), and stretching movements were used to cool down at the end of the training session. The amount of supplements is shown in Table 2 [19, 20].

Table 2. Consumption of supplements

Groups		**Six weeks of weight training with supplement**		**The 7th and 8th week of non-training**
		Days when subjects practiced	**The days when subjects did not practice**	**Two weeks of non-training**
HMB group		3 g daily (1 g after lunch,1 g before exercise, and 1 g after exercise)	1 g daily after breakfast	1 g daily after breakfast
Cr group	**In the first 5 days as a daily load of 20 g (5 g in the morning fasting, 5 g before exercise, 5 g after exercise, and 5 g at bedtime)**	15 g daily (5 g in the morning fasting, 5 g before exercise, and 5 g after exercise)	5 g daily (morning fasting)	5 g daily (morning fasting)
HMB+ Cr group		HMB supplement 3 g per day (1 g after lunch, 1 g before exercise, and 1 g after exercise) and Cr supplement 15 g daily (5 g in the morning fasting, 5 g before exercise, and 5 g after exercise)	HMB supplement 1 g daily after breakfast and Cr supplement 5 g daily (morning fasting)	HMB supplement 1 g daily after breakfast and Cr supplement 5 g daily (morning fasting)

The present study consisted of three stages: pre-test, post-test (end of the 6 weeks), and 2 weeks of non-training (end of the 8th week). The goal of the 2 weeks of non-training was that the subjects did not perform any training protocol after a 6-week period. They took only supplements during the two weeks to see whether the effect of supplementation with exercise or only taking supplements without exercise alone was more effective on blood biochemical factors. To prevent the possible effects of interfering variables, subjects were advised to avoid taking other supplements, caffeinated substances, and drugs during the supplementation period and not to change their diet during the test period. The means of the blood biochemical factors of (total Chol., TG, LDL, and HDL) were measured in the subjects by taking blood samples three times. The first blood sample was taken on the morning of the test day before the start of the training program and supplementation, and the second blood sample was taken on the morning of the day after the last training session (at the end of the 6 weeks). The third blood sample was taken on the morning of the day at the end of the 8^{th} week after 2 weeks of non-training. Thus, subjects were asked to fast for 12 h and avoid any exercise 24 h before blood sampling. Ten cc of the blood was taken from all subjects and was tested after centrifugation and separation of serum for laboratory tests to measure Chol., TG, LDL, and HDL. Data were analyzed using descriptive statistical tests. The normality of variances and data was evaluated using Machley and Kolmogorov-Smirnov (K-S) tests, respectively. The hypotheses were tested by inferential statistical tests using two-way repeated-measures analysis of variance (ANOVA) (Table 3). In the case of significant differences, the Bonferroni post hoc test was used for binary comparisons.

RESULTS AND DISCUSSION

The results of repeated-measures ANOVA showed that the interaction effect of the groups (weight training group and supplementation with Cr, HMB, and HMB + Cr) was significant on total Chol., TG, HDL, and LDL levels (p = 0000) (Table 3).

Table 3. Repeated measures ANOVA statistical test results

Variable	F	Significant level
Measurement of Total Cholesterol × group	7.820	0.000
Measurement of Triglyceride × group	6.289	0.000
Measurement of HDL × group	22.295	0.000
Measurement of LDL × group	8.997	0.000

The results of ANOVA showed that there was no significant difference in the amount of cholesterol between the groups after the test and 2 weeks of non-training ($p \geq 0.05$) (Table 4).

Table 4. Comparison of mean cholesterol in post-test and 2 weeks of non-training between weight training, Cr, HMB, and HMB + Cr groups.

Measurement Session	groups		Mean Difference	Standard error	Significant level	Assurance distance	
						Min	max
Post test	Weight training	HMB+ Cr	4.375	7.226	1.000	-16.139	24.889
Post test	Cr	Weight Training	3.125	7.226	1.000	-17.389	23.639
Post test	Cr	Weight Training	7.500	7.226	1.000	-13.014	28.014
Post test	HMB	Cr	-9.500	7.226	1.000	-30.014	11.014
Post test	HMB	HMB +Cr	-12.625	7.226	0.549	-33.139	7.889
Post test	HMB	HMB +Cr	-5.125	7.226	1.000	-25.639	15.389
2weeks of non Training	Weight Training	HMB +Cr	3.625	7.194	1.000	-16.797	24.047
2weeks of non Training	Cr	Weight Training	4.000	7.194	1.000	-16.422	24.422
2weeks of non Training	Cr	HMB +Cr	7.625	7.194	1.000	-12.797	28.047
2weeks of non training	HMB	Weight Training	-9.000	7.194	1.000	-29.422	11.422
2weeks of non training	HMB	Cr	-13.000	7.194	0.489	-33.422	7.422
2weeks of non training	HMB	HMB +Cr	-5.375	7.194	1.000	-25.797	15.047

The results of ANOVA indicated that there was no significant difference in the TG content between the groups after the test and 2 weeks of non-training ($p \geq 0.05$) (Table 5).

Table 5. Comparison of mean TG in post-test and 2 weeks of non-training between weight training, Cr, HMB, HMB + Cr groups

Measurement Session	Groups		Mean difference	Standard error	Significance level	Assurance distance	
						Min	Max
Post - test	Weight Training	HMB +Cr	6.000	8.225	1.000	-17.350	29.350
Post - test	Cr	Weight Training	-2.125	8.225	1.000	-25.475	21.225
Post - test	Cr	HMB +Cr	3.875	8.225	1.000	-19.475	27.225
Post - test	HMB	Weight Training	6.375	8.225	1.000	-16.975	29.725
Post – test	HMB	Cr	8.500	8.225	1.000	-14.850	31.850
Post – test	HMB	HMB +Cr	12.375	8.225	0.862	-10.975	35.725
2weeks of non- training	Weight Training	HMB +Cr	8.250	8.452	1.000	-15.744	32.244
2weeks of non- training	Cr	Weight Training	-1.375	8.452	1.000	-25.369	22.619
2weeks of non -training	Cr	HMB+ Cr	6.875	8.452	1.000	-17.119	30.869
2weeks of non- training	HMB	Weight Training	4.000	8.452	1.000	-19.994	27.994
2weeks of non -training	HMB	Cr	5.375	8.452	1.000	-18.619	29.369
2weeks of non -training	HMB	HMB +Cr	12.250	8.452	0.950	-11.744	36.244

The results of ANOVA revealed that there was no significant difference in the LDL values of the subjects after the test and 2 weeks of non-training ($p \geq 0.05$) (Table 6).

Table 6. Comparison of mean LDL in post-test and 2 weeks of non-training between weight training, Cr, HMB, and HMB + Cr groups

Measurement Session	Groups		Mean difference	Standard Error	Significance Level	Assurance distance	
						Min	Max
Post – test	Weight Training	HMB +Cr	4.750	5.355	1.000	-10.454	19.954
Post – test	Cr	Weight Training	6.125	5.355	1.000	-21.329	9.079
Post – test	Cr	HMB +Cr	-1.375	5.355	1.000	-16.579	13.829
Post – test	HMB	Weight Training	-8.625	5.355	0.711	-23.829	6.579
Post – test	HMB	Cr	-2.500	5.355	1.000	-17.704	12.704
Post – test	HMB	HMB +Cr	-3.875	5.355	1.000	-19.079	11.329
2weeks of non- training	Weight Training	HMB +Cr	3.625	5.534	1.000	-12.086	19.336
2weeks of non- training	Cr	Weight Training	-6.250	5.534	1.000	-21.961	9.461
2weeks of non- training	Cr	HMB+ Cr	-2.625	5.534	1.000	-18.336	13.085
2weeks of non- training	HMB	Weight Training	-6.875	5.534	1.000	-22.586	8.836
2weeks of non- training	HMB	Cr	-0.625	5.534	1.000	-16.336	15.086
2weeks of non -training	HMB	HMB +Cr	-3.250	5.534	1.000	-18.961	12.461

According to the results of ANOVA, significant differences were observed in the post-test between weight training and HMB groups (p = 0.000), weight training and HMB + Cr groups (p = 0.002), Cr and HMB groups (p = 0.000), and Cr and HMB + Cr groups (p = 0.001). There were significant differences in the HDL values of the subjects between the weight training and HMB groups (p = 0.000), weight training and HMB + Cr groups (p = 0.003), Cr and HMB groups (p = 0.000), and Cr and HMB + Cr groups (p = 0. 001) in 2 weeks of non-training (Table 7).

Table 7. Comparison of mean HDL levels in post-test and 2 weeks of non-training between weight training, Cr, HMB, and HMB + Cr groups

Measurement Session	Groups		Mean difference	Standard Error	Significance Level	Assurance distance	
						Min	Max
Post – test	Weight Training	HMB +Cr	-6.625	1.630	0.002	-11.253	-1.997
Post – test	Cr	Weight Training	-0.250	1.630	1.000	-4.878	4.378
Post – test	Cr	HMB +Cr	-6.875	1.630	0.001	-11.503	-2.247
Post – test	HMB	Weight Training	8.750	1.630	0.000	4.122	13.378
Post – test	HMB	Cr	9.000	1.630	0.000	4.372	13.628
Post – test	HMB	HMB +Cr	2.125	1.630	1.000	-2.503	6.753
2weeks of non- training	Weight Training	HMB +Cr	-6.125	1.532	0.003	-10.473	-1.777
2weeks of non -training	Cr	Weight Training	-0.375	1.532	1.000	-4.723	3.973
2weeks of non- training	Cr	HMB+ Cr	-6.500	1.532	0.001	-10.848	-2.152
2weeks of non -training	HMB	Weight Training	7.500	1.532	0.000	3.152	11.848
2weeks of non- training	HMB	Cr	7.875	1.532	0.000	3.527	12.223
2weeks of non -training	HMB	HMB +Cr	1.375	1.532	1.000	-2.973	5.723

Based on the results obtained in the post-test, a significant decrease was observed in the changes of mean Chol. levels of the subjects in the groups supplemented with Cr, HMB, and HMB + Cr. In 2 weeks of non-training (end of the 8th week), a significant decrease was observed in the changes of mean Chol. levels of the subjects in the groups supplemented with HMB and HMB + Cr compared to the pre-test. In confirmation of our results, the exercise not only lowers total blood Chol. but also increases the part of Chol., called HDL-C lipoprotein, and decreases the other part, i.e. LDL-C lipoprotein [7]. The relationship between trunk muscle resistance training and serum lipids was investigated in 27 subjects, and it was concluded that the program significantly reduced Chol. levels [3]. The effect of HMB supplementation was investigated along with selected resistance training on some blood biochemical factors in 16 volunteer wrestlers. The experimental group presented a significant reduction in total Chol. [21].

Based on the results obtained in the post-test, a significant decrease was observed in the mean TG changes of the subjects in the weight training group and those in Cr, HMB, and HMB+Cr supplementation groups. In 2 weeks of non-training, a significant increase in the mean TG of subjects was observed in the Cr-supplement group compared to the post-test. In 2 weeks of non-training, a significant decrease was also found in the mean TG changes of the subjects in Cr, HMB, and HMB + Cr supplementation groups compared to the pretest. A comparison between groups also showed no significant differences in Col., TG, and LDL concentrations of the subjects in the four groups of weight training and Cr , HMB, and HMB + Cr supplementation groups in the post-test and 2 weeks of non-training. In confirmation of these results, the relationship between torso muscle resistance training and serum lipids was investigated in 27 subjects, and it was concluded that the program not only significantly reduced TG but also reduced fat in the abdomen and other areas of the body that were affected by exercise [3]. In contrast, the effect of HMB supplementation along with selected resistance training on some blood biochemical factors in 16 volunteer wrestlers indicated an increase in TG levels of the subjects in both experimental and control groups, but this increase was not significant [21].

Based on the results obtained in the post-test, a significant decrease in the mean LDL changes of the subjects was recorded in the HMB and HMB + Cr supplement groups. In 2 weeks of non-training, the subjects in HMB and HMB + Cr supplement groups showed a significant decrease in the mean LDL compared to the pretest. A comparison between groups revealed no significant differences in Chol., TG, and LDL measurements of the subjects in the four groups of weight training and Cr, HMB, and HMB + Cr supplementation groups in the post-test and 2 weeks of non-training. In confirmation of these results, the effect of supplementation of HMB along with selected resistance training was investigated on some blood biochemical factors of 16 volunteer wrestlers, and the results showed a decrease in LDL of subjects in both experimental and control groups, but this decrease was not significant [21]. The examination of HMB supplementation in cardiovascular damage indicated a reduction in LDL Chol. [22].

Based on the results obtained in the post-test, a significant increase was observed in the mean changes in HDL of the subjects in the groups supplemented with HMB and HMB + Cr. In two weeks of non-training, a significant decrease was observed in the mean changes in HDL of the subjects in the HMB and HMB + Cr supplement groups compared to the post-test. Moreover, the mean HDL of the subjects significantly decreased in the HMB and HMB + Cr supplementation group in 2 weeks of non-training compared to the pretest. A comparison between the groups also showed that significant differences between the weight training and HMB groups, weight training and HMB + Cr group, Cr and HMB groups, and Cr and HMB + Cr groups in the post-test. In 2 weeks of non-training, there were significant differences between the weight training and HMB groups, weight training and HMB + Cr groups, Cr and HMB groups, and Cr and HMB + Cr groups. In confirmation of these results, the exercise not only lowers total blood Chol. but also increases the part of Chol. called HDL-C lipoprotein and the other part, i.e. LDL-C lipoprotein [7].

CONCLUSIONS

This article aimed to evaluate the effect of 6 weeks of weight training with HMB and Cr supplementation and 2 weeks of non-training on TG, LDL, HDL, and Chol. levels in male powerlifting athletes. The results showed that HMB + Cr supplementation in post-test and 2 weeks of non-training increased HDL and decreased Chol., TG, and LDL. In the HMB + Cr supplementation group, however, the effect of HMB + Cr supplementation was significantly increased on HDL compared to the Cr supplementation and the weight training groups. Moreover, HMB was more effective in increasing HDL in the HMB + Cr supplementation group than Cr.

ACKNOWLEDGEMENTS

The present study is the result of a doctoral dissertation at the Islamic Azad University of Damghan. The authors appreciate all those who contributed to this research.

Conflict of interest

All authors declare no conflict of interest in this study.

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