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Investigating the Interactive Effect of HMB-FA and Extrovert Physical Exercise on LDH Muscular Damage in Mature Male Rats

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ABSTRACT

Lactate is a glycolysis product that can be produced continuously by large cells at rest and even under sufficient oxygen conditions. It is suggested for extensive research to be conducted on the effects of HMB supplementation in human and animal samples. The aim of this study was to investigate the interactive effects of eccentric exercise and supplement HMB-FA on LDH in adult male rats. The 24 adult male rats split into four groups: control group (n=6), HMB (n=6), exercise (n=6) and Sport and HMB (n=6). After three familiarity session for experimental groups with training and obtain their 1RM, to supplement daily was administered weekly for two weeks. Then of course full load, eccentric resistance exercise was carried out. Upon completion of the exercise protocol blood of rats was carried out using LDH was measured by ELISA. Finally, data were analyzed using statistical software SPSS version 18. The finding no significant effect of supplementation with HMB-FA and eccentric resistance exercise on these variables have not found and the Conclusions this study found that in contrast to the results obtained in previous studies HMB-FA supplementation and eccentric resistance exercise no significant impact on the levels of LDH in adult male.

Key words: HMB-FA, Physical Exercise, LDH, Male Rats.

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INTRODUCTION

Athletes of various sports disciplines use various exercises to increase physical fitness nowadays; one of the exercises that practically all athletes use is resistance training [1]. This type of workout is done to improve performance, achieve peak performance, increase tonus and muscle strength, hypertrophy, and enhance body fitness [2]. In most cases, muscle damage in sports activities results from an inappropriate exercise activity. The obvious characteristic of this type of contraction is to exert double pressure on the muscle, soft tissues and, as a result, muscle damage in the first place. The development of damage and extrarenal muscle inflammation, the severity of which depends on the duration of the activity, the levels, or the condition of the muscle training practice, has been reported in several studies[3]. Repeated exposure to overload causes tissue to stimulate overload, but excessive exercise overload causes soft tissue damage. This issue increases during resistance exercises, resulting in athletes needing more retrieval to increase exercise times to improve their performance. Typically, resistance training programs or competitive exercises do not allow the full recovery between exercise sessions or races to the athlete, resulting in catabolic conditions in muscle tissue [4]. Eccentric contractions do not occur only during sports activities, but also in daily activities, such as coming down from stairs or lowering a heavy load from a height. Although eccentric contraction in terms of metabolism requires less energy than other activities, this kind of contraction causes skeletal muscle microstructure, stronger inflammatory response, and also a greater

proportion of oxidative stress compared to intrinsic activity, including activities that reduce muscle length during contraction [5]. The development of damage and extrarenal muscle inflammation, the severity of which depends on the duration of the activity, the levels, or the condition of the muscle training practice, has been reported in several studies[6]. Damage and development of muscle inflammation due to eccentric activity have been reported in several studies, whose severity is dependent on intensity and duration of activity, as well as levels or muscle training [6]. Creatine kinase and lactate dehydrogenase are the most common signs of muscle damage that may change after severe physical activity [7]. Researchers have shown that aspartate aminotransferase, creatine phosphokinase and lactate dehydrogenase are important enzymes for muscle damage. LDH is an enzyme that is widely found in the cytoplasm of all tissues of the body with different concentrations, and it speeds up the transfer of Pyruvic acid to Lactic acid or vice versa in an anesthetic glycolysis membrane. The Lactate dehydrogenase enzyme is a tetramer and consists of four polypeptide chains. Changes in this enzyme occur later than the CPK, and usually increase gradually from 24 to 48 hours after the test. There is naturally a small amount of lactate dehydrogenase in plasma, a significant amount for which is released in the blood following a myocardial infarction, liver disease, or muscle and tissue damage. The use of nutritional and dietary supplements can also be a good way to prevent cell damage and increase inflammatory markers [8- 10]. Beta-hydroxy beta-methylbutyrate (HMB) is, also, one of the supplements that help to maintain muscle mass by reducing cell protein degradation and providing cell membrane cholesterol synthesis precursors and it functions as a potential candidate for increasing anabolic hormones, mass development and muscle growth under various conditions. This substance is the result of chemical decomposition of leucine amino acid which is commonly produced in the liver. There are several studies on HMB supplementation that have contradictory results. Considering that previous research has been carried out mostly on humans, doing similar research on male rats can have interesting and desirable results. For this purpose, in order to clarify the use of this supplement and its effect on muscle damage, the present study has been conducted to examine the complementary effects of HMB-Fa, which is more rapidly absorbed in comparison to HMB and HMB-Ca.

MATERIAL AND METHODS

The present semi-experimental study, which is based on levels of monitoring and control, is, also, a laboratory research; the study population included Sprague-Dawley male rats and two variables were considered and analyzed, with two weeks of HMB-FA supplementation being the independent variable and the level of lactate dehydrogenase as the dependent variable. For the maintenance of the cells, the room temperature and standard brightness were measured and daily dietary intake and body weight of the rats were measured every six days. Measuring the weight of the rats on the scales was started on the first day of the study and recorded using the Bio space scale of the BSM 370 model. The rats were randomly divided into four groups of control, HMB, exercise, and exercise with HMB. Initially, the experimental groups received three sessions of familiarization by coming down from the ladder and gaining 1RM with a 55% of their body weight external object tied to their tails; is the rats climbed the ladder successfully, the weight would increase more and more. In the event of a successful run, we increased the weights to 35 grams each time until the rats failed and did not have the ability to climb the ladder. Pre-failure weight was considered as 1RM. The rest between the repetitions was 2 minutes, and the ladder was adjusted to a height of 1 meter and an adjustable slope and the distance between fences was 1 centimeter. After the familiarization stage, two weekly supplemental intakes were performed on a daily basis. In experimental groups, 255 mg/kg body weight/day, supplementation was dosed in 1 ml of distilled water and gavaged to rats. After completing the loading period, one session of severe external resistance was performed in form of coming down from the ladder with a slope of 80% and the closure of 120% 1RM at the back of the tail. Each rat was placed on top of the ladder by the examiner and it was supposed to come down eight times from the ladder in each set and the protocol was completed if three sets of eight repetition for each set was done. The protocol was terminated if the rat failed to finish the resistance session even after three breaks between the sets. Each rat rested for 2 minutes between repetitions. Blood samples were collected from the left ventricle of the rats (5cc) using Venoject Needle after anesthetizing them. Then, blood samples were centrifuged in the laboratory and the level of creatine kinase and lactate dehydrogenase was measured through ELISA method and the obtained data was analyzed using spss18 and chi-square

Table 1: Different levels of LDH in four different conditions

Variable	Research conditions	Mean	SD
Lactate Dehydrogenase	Basic	1537	273.1
	HMB-FA supplement	1001.6	708.05
	Extroverted resistance exercises	1068.3	375.6
	HMB-FA supplements plus resistance training	1372	632.5

Table 2: The results of Shapiro–Wilk test

Variable	Groups	D	Df	Sig.
Lactate Dehydrogenase	Basic	0.999	3	0.939
	HMB-FA supplement	0.881	3	0.327
	Extroverted resistance exercises	0.807	3	0.132
	HMB-FA supplements plus resistance training	0.778	3	0.063

Tests [11-13]. P<0.05 was considered the significance level.

RESULTS

24 Sprague-Dawley male rats were examined in the present study. The variables of LDH levels were described in four basic conditions of without exercise and no supplementation, complement supplementation, HMB-FA supplementation, extrinsic resistance exercises, and HMB-FA complement supplementation with extroverted resistance training using mean and standard deviation; the highest mean, 1537 IU/L, turned out to be related to the base conditions (Table 1). The results of Shapiro-Wilk test showed that LDH levels were naturally distributed in four baseline conditions of no exercise and no supplementation, HMB-FA supplementation, extroverted resistance exercises, and HMB-FA supplementation plus extroverted resistance training (P<0.05)(Table 2). Also, the results of two variable factor analysis showed that the main effect of HMB-FA supplementation on LDH levels in adult male rats was not significant (P>0.05). in other words, without paying attention to the frequency of exercise, there is no significant difference between the mean levels of LDH in adult male rats in complementary and control conditions. Also, the effect of extrinsic resistance training on LDH levels in adult male rats was not significant (P>0.05). In addition, the interactive effect of extrinsic resistance training and supplementation on LDH levels in adult male rats was not significant (P>0.05). In other words, the complementary effect on the LDH of adult male rats is the same in the resistance training levels.

DISCUSSION

Many sports supplements are used by athletes with the claim that they improve muscle strength and body composition and complement the body [14]. Fatigue is the most important factor in the ability of a person to perform better, especially during short periods of exercise. Excessive fatigue usually limits the performance of the athlete and delays the desired outcome [15]. One of the causes of local fatigue is the accumulation of lactic acid in active muscles and the concentration of hydrogen ion in the blood [16]. Increasing lactic acid after intensive activities due to impaired energy production and desired muscle contractions have led researchers to find ways to release LDH faster and reduce its production in the bloodstream [17]. β-Hydroxyβ-Mehylbutyrate=HMB metabolite is derived from leucine, the safety of which is not still scientifically proven; there have been also few studies about the effects of these supplements on health indexes in animals which bhave, to some extent, proven the safe effects of these supplements on animals[18, 19 and 20]. The present study was conducted to investigate the intercative effect of HMB-FA and extrovert physical exercise on LDH muscular damage in mature male rats. As stated above, extrinsic resistance training can increase LDH enzyme by causing muscle damage, but HMB supplementation, while maintaining the integrity of the cell membrane during injury, prevents the introduction of cell contents and enzymes into the plasma. Nissen, Smith, and Thomson studies have claimed that the use of HMB supplementation in aerobic activities reduces LDH levels and muscle damage in short and long term[21, 22 and 23]. However, the results of the present study showed that HMB-FA supplementation, as well as extrinsic resistance training, had no significant effect on LDH levels in adult male rats (P>0.05). Although the interactive effect of these two variables on the LDH level is large and previous studies have confirmed the effect of this supplement on the reduction of LDH levels in humans, this hypothesis

is rejected in the present study which was conducted on male rats, with the reason, in all probability, being limited and small study population. In fact, given that resistance training increases the enzyme and supplementation of HMB reduces its levels, the interactive use of these two supplements may in turn neutralize the effects of each one on the changes in the enzyme. In recent studies, the effect of resistance training indicated increased LDH levels[19], but according to the results of this study, extrinsic resistance training did not have a significant effect on LDH levels in adult male rats, which, according to a small number of samples, rejecting this hypothesis necessitates more research. The results of Faramarzi *et al.,* (2012) examined the effects of β-hydroxy-β-methylbutyrate on kidney parameters and body composition in untrained males after 8 weeks of combination resistance training, showed that HMB supplements increased the net mass and the strength of one repeat Maximum body fat loss in non-athlete subjects. However, there is no harmful effect on renal function during the period spent in this study [24]. Contrary to the results of the current study, Kornasio *et al.,* (2009) acknowledged that the number of mycoblastic myoblasts derived from satellite cells in the presence of HMB decreased, and HMB increased the amplification of myogenic cells[25]; these findings suggest that HMB inhibits protein excretion and apoptosis, and thus maintains the satellite cells that are essential for muscle reconstruction.

CONCLUSION

The results of the present study showed that HMB supplements and extrinsic resistance training had no significant effect on LDH levels in male rats, which is not consistent with the results of previous studies on humans. It is suggested that extensive studies be conducted on the effects of HMB supplementation in human and animal samples.

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