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The effects of glutamine on serum LDH and CK in rats following a session of resistance activity

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Abstract

Introduction: Muscle pain after resistance activities, which occurs due to the destruction of sarcolema, increases the serum levels of LDH and CK enzymes as indicators of muscle damage. The study's results have shown that the use of food supplements such as glutamine can be useful in reducing the serum indicators of muscle damage. The present study aims to determine the effect of glutamine supplementation on serum indicators of muscle damage, including the serum levels of LDH and CK enzymes following a session of resistance activity (RA).

Material & Methods: The present experimental study was carried out using an animal model in the form of a 3 group research design with a control group. To this end, 30 adult male Wistar rats (six-week-old) were kept under controlled conditions for 2 weeks and were then divided into three equal groups, including control, and resistance activity with/ without glutamine supplementation. The glutamine supplementation group received the prepared emulsion by gavage of 200 mg/kg of body weight. After five days, both experimental groups participated in a session of RA (namely, climbing a smooth ramp with 1.5 meters height and an 85° decline) with 4 sets, 5 repetitions, 30 seconds of rest between repetitions, and 2 minutes of rest between sets. The initial load was equal to 50% of the rats' body weight. One-way analysis of variance and Bonferroni's post hoc test were used at a significance level of $p \geqslant 0.05$.

Results: The levels of CK and LDH enzymes were different in groups. Five-day glutamine supplementation before performing a session of resistance activity can cause a lower increase in the serum levels of CK and LDH enzymes (P=0.001) as serum indicators of muscle damage, which indicates the protective effect of glutamine in maintaining the integrity and structure of cell membranes.

Conclusion: Our result suggests that glutamine consumption in animal samples can reduce muscle damage after progressive strength activity. This property can be used to benefit from the possible protective effects of glutamine in maintaining the structure and integrity of the muscle fiber cell membrane and reducing fiber damage.

1. Introduction

Body tissues are damaged as a result of mechanical and metabolic factors caused by long and high-intensity training. High-intensity physical activity, despite its various benefits for health, can be due to the increase in oxidative stress caused by the increase in metabolism and the production of reactive oxygen and nitrogen species (free radicals), and the creation of very high mechanical stress beyond the ability and tolerance of muscle structures, leading

to possible sarcomere damage. Mechanical factors during exercise cause damage to sarcomere membranes through sarcomere stretching. The production of metabolic factors during exercise, such as the formation of free radicals and excessive accumulation of calcium, causes cell damage. The high consumption of oxygen during exercise leads to an increase in the production of reactive species due to the increased activity of the electron transport chain, which subsequently causes damage to the cell membrane. Cell destruction occurs due to direct and/or indirect damage to the muscle membrane and leads to the penetration of the intracellular components into the extracellular fluid (1). Destruction of the cell membrane causes the instability of the sarcomere and the subsequent release of enzymes and intracellular contents such as lactate dehydrogenase (LDH) and creatine kinase (CK) into the blood serum, which are considered serum indicators of muscle damage(2, 3) LDH catalyzes the two-way reaction of converting pyruvic acid to lactic acid through the oxidation-reduction reaction of nicotinamide adenine dinucleotide (4). A significant increase in plasma levels of LDH occurs with a small amount of skeletal muscle damage or destruction caused by high-intensity exercise and muscle trauma (5). Creatine phosphokinase or creatine kinase (CK) catalyzes the two-way reaction between adenosine triphosphate and creatine, and its highest activity is in skeletal muscles (6). LDH and CK can be used alongside them to build a more complete picture of physiological disturbances. Alongside an increase in circulating muscle proteins, there is also a strong inflammatory response to eccentric exercise that contributes to exercise-induced muscle damage (EIMD) and is essential for regulating adaptation to resistance exercise. This response is highly orchestrated and involves both immune cells and associated cytokines (IL-1, IL-6, IL-10, TNF-α). The accumulation of inflammatory cells in the muscle tissue is considered an important indicator of EIMD (7).

One of the ways to deal with the complications of sports injuries is to take anti-inflammatory and antioxidant food supplements (8, 9). One of the supplements used is the amino acid glutamine, which is very important in skeletal muscles to maintain protein levels, immune system function, and glucose-glycogen metabolism. Glutamine dipeptide supplementation increases ammonia and glycogen concentrations in skeletal muscle, and glutamine plus alanine in their free form prevents muscle ammonia increase during resistive exercise training (10). Glutamine is the most abundant free amino acid in the human body, which constitutes 50-60% of the total free amino acid reserves of skeletal muscles and about 20% of the total amino acid reserves of plasma. The body can produce the amount of glutamine it needs as a non-essential amino acid, but in heavy, high-intensity, and long-term exercises, the levels of glutamine decrease, in which case more glutamine is needed to be received as an essential amino acid through nutrition or food supplements (11). Glutamine not only leads to an increase in protein synthesis in muscles but also reduces the intensity and amount of sarcomere protein breakdown that occurs due to high-intensity training (12, 13). L-glutamine is the most abundant amino acid in the body, and studies have shown that its availability is critical to the glutathione (GSH)-mediated antioxidant defense (14, 15). GSH (L-γ-glutamyl-L-cysteinyl-glycine) is quantitatively the most important non-enzymatic antioxidant and scavenger, providing protection against oxidative stress (16) cell damage and inflammation (17). As a result, the present study aimed to determine the effect of glutamine supplementation on serum indicators of muscle damage, including serum levels of LDH and CK enzymes in male Wistar rats after a session of resistance activity.

2. Materials and methods

The present experimental research was conducted with the aim of determining the effect of glutamine supplementation on serum indicators of muscle damage in adult male Wistar rats after a session of resistance activity using an animal model in the form of a multi-group research design with a control group. In the present study, 30 six-week-old adult male Wistar rats with an average weight of 120 grams were prepared and kept in controlled conditions for two weeks in order to familiarize and adapt to the living environment, nutritional, and training conditions. The rats were then divided into three equal groups, including (1) control (which did not participate in the program of taking glutamine supplements and didn't take part in a session of resistance activity, but were sampled to determine the basic values of the research variables), (2) glutamine supplementation and resistance activity (which showed the amount and manner of changes in the serum indicators of muscle damage in adult male Wistar rats after five days of glutamine supplementation and one session of resistance activity, and (3) resistance activity (which showed the amount and manner of changes in the serum indicators of muscle damage in adult male Wistar rats after one session of resistance activity). During this period, all rats had access to standard food and water. For two weeks, the rats were introduced to the resistance activity on the ramp. During this period, the amount of electric shock was constant at 0.1 mV.

The level of serum CK enzyme activity was determined by a chemical colorimetric method based on Jaffe with a sensitivity of 1 U/L and a coefficient of 1.6% (CK colorimetric kit, Pars Azmoun, Tehran, Iran). The activity of the LDH enzyme was determined by the enzyme colorimetric method with U/L5 sensitivity and a change factor of 1.2 (LDH colorimetric kit, Pars Azmoun, Tehran, Iran).

Homogeneity of variance and residual normality assumptions were tested by using the Levene's and Shapiro–Wilk tests respectively. One-way analysis of variance and Bonferroni's statistical tests were used to determine the significant difference between the tested groups. The significance level in all tests was $p \geqslant 0.05$. The research process was performed and implemented according to the following protocol (Table 1).

Table 1. *Research implementation protocol*

Group	Weeks (1-2)	Week 3 (Day)						
		1	2	3	4	5	6	
							Activity	+ 6 – 8 h
Control Glutamine supplementation and resistance activity resistance activity	Maintenance in controlled conditions (adaptability with the living environment, nutrition, and training conditions). Weight measured on day 14	-------- Taking glutamine (200 mg/kg) supplement --------					-------- Performance of one session of resistance activity	Measurement of the research variables

2.1. Glutamine Supplementation

The glutamine supplementation group received the prepared emulsion composition at the rate of 200 mg/kg of body weight by gavage twice a day for five days (The glutamine powder dissolved in 100 cc of distilled water). L-glutamine Viva Power 100g powder under the license of Vitafarmed is produced by Faryab Daru Company, Karen Pharmaceutical Company (Iran) under the license of Switzerland and delivered in plastic packs containing pure powder and 100% L-glutamine supplement. In the current study, glutamine amino acid was used in the form of white powder with a purity of more than 99.5% and solubility in water at 20°C in a transparent and colorless form with a bioavailability of more than 99.5%.

2.2. Resistance activity

Both experimental groups participated in one session of resistance activity (namely, climbing a smooth ramp with one and a half meters height and 85° incline) with four sets, five repetitions, thirty seconds of rest between repetitions, and two minutes of rest between sets. The initial load was considered equal to 50% of the rats' body weight. Then, at the beginning of each set, 10% of the rats' body weight was added to the initial load, so that at the end of the fourth set, each rat carried 80% of its body weight.

2.3. Blood sampling

Based on the predetermined schedule, all rats were anesthetized, killed, and operated on 6 to 8 hours after the resistance training session. The rats were anesthetized by intraperitoneal injection of ketamine (90 mg per kg body weight) and xylazine (10 mg per kg body weight). Then they were immediately killed and operated on by experienced specialists. Given the aim of the present study, two miles of blood were taken from the heart tissue of the dissected rats using two-milliliter syringes. In order to separate the serum from the blood, the blood samples taken were centrifuged at a temperature of 4° C at a speed of 5000 rpm for ten minutes.

3. Results

The serum levels of LDH [F (2, 27) = 15.208, P⩾ 0.001] and CK [F (2, 27) = 35.245, P⩾ 0.001] variables in the three groups were different (Table 2). The results of Bonferroni's statistical test showed that this difference was significant among all groups (Table 3).

Performing one session of resistance activity causes micro-muscle damage, which results in an increase in the serum levels of LDH and CK enzymes due to the destruction of the sarcomeric membrane and the release of these enzymes into the blood serum. This result can be postulated from the statistically significant difference between the average levels of LDH and CK enzymes in control and one-session resistance activity groups, which is accompanied by an increase of 55.33% in LDH and 60.45% in CK. In addition, the increase in the serum levels of LDH and CK enzymes in the one-session activity and glutamine supplementation group was 31.84% and 18.14%, respectively, which was different from the one-session resistance activity group without glutamine supplementation. On the other hand, five-day supplementation of glutamine caused a significant decrease in the serum levels of LDH and CK enzymes by 23.50% and 42.30%, respectively, which was different from the one-session resistance activity group without glutamine supplementation.

Table 2. *The results of the one-way ANOVA to compare the LDH and CK enzymes*

variables	Source of variations	Sum of squares	df	Mean square	F value	P
LDH (IU)	Between-group	113206.284	2	56603.142	15.208	≤ 0.001*
	Within group	100492.164	27	3721.932		
	Total	213698.448	29	-		
CK (IU)	Between-group	199920.707	2	99960.353	35.245	≤ 0.001*
	Within group	76576.239	27	2836.157		
	Total	276496.946	29	-		

Table 3. *The results of Bonferroni's post hoc test to compare the levels of LDH and CK enzymes*

Serum LDH	Control	resistance activity
resistance activity	P≤0.001	-
resistance activity and glutamine	0.001	P≤0.001
Serum CK		
resistance activity	P≤0.001	-
resistance activity and glutamine	0.021	P≤0.001

4. Discussion

A five-day period of glutamine supplementation before performing one session of resistance activity can cause a lower increase in the serum levels of LDH and CK enzymes as serum indicators of muscle damage, which indicates the protective effect of glutamine in maintaining the integrity of cell membrane and reducing the amount of damage to muscle fibers.

In a review article (18) emphasized the use of nutritional strategies and supplementation to prevent and reduce exercise-induced muscle damage. Naclerio et al. (2015) in an article entitled " A multi-ingredient containing carbohydrate, proteins L-glutamine and L-carnitine attenuates fatigue perception with no effect on performance, muscle damage or immunity in soccer players " investigated the effects of consuming a multi-ingredient supplement (53 grams of carbohydrates, 14.5 grams of whey protein, 5 grams of glutamine, 1.5 grams of L-carnitine-L-tartrate) on intermittent performance, fatigue perception, safety indicators, damage indicators, and functional and metabolic markers. Muscle damage indicators including CK and myoglobin increased after high-intensity activity in all groups. Of course, this increase in the supplementation group was less than in other groups, which indicates the positive and beneficial effect of using these combined supplements on muscle damage indicators (19). Raizel & Tirapegui (2018) in a review article studied the role of glutamine in the recovery of leg muscles following resistance training. Taking oral glutamine can reduce the damage and inflammation caused by high-intensity and fatiguing aerobic training. However, its effects on muscle recovery in resistance training are not clear (20). Raizel et al.'s (2019) research entitled "Glutamine as an anti-fatigue amino acid in sports nutrition" showed that glutamine supplementation improved some fatigue markers such as increasing glycogen synthesis and reducing ammonia accumulation, but this intervention did not increase physical performance. Therefore, despite improving some fatigue parameters, glutamine supplementation appears to have

limited effects on performance (20). Nemati et al. (2019) studied the effect of glutamine supplementation on oxidative stress after exhaustive exercise. The results showed that the serum level of oxidative stress and muscle damage indicators decreased significantly in the supplementation group, yet the serum level of antioxidant indicators increased in the glutamine supplementation group (21). Regarding the effect of glutamine supplementation on the serum levels of some inflammatory factors and oxidative stress, Mohajeri et al. (2021) suggested that following 5 days of glutamine supplementation, these indicators were significantly reduced (22). Córdova-Martínez et al. (2021) studied the effect of glutamine supplementation on muscle damage biomarkers in professional basketball players. The glutamine-supplemented group showed lower blood levels of aspartate transaminase, creatine kinase, and myoglobin, indicating less muscle damage compared to the placebo. The results showed that glutamine could help reduce exercise-induced muscle damage (23). Increased intramuscular glutamine levels have been directly linked to influencing muscle cell volume (24), which enhances protein synthesis, and increases muscle size. By increasing muscle mass, the contractile force of a muscle can be increased (25). Rahmani-Nia et al. (2012) studied the effect of glutamine supplementation on delayed muscle soreness and muscle electrical activity after eccentric contractions in untrained men. There was no significant difference in creatine kinase and electromyographic activity between the two groups (26). Najarzadeh et al. (2014) studied the effect of consuming a glutamine supplement on muscle damage indicators after eccentric resistance activity. In none of the measurement times, glutamine supplementation had a significant difference in reducing the amounts of CK and LDH (27).

Moini Najaf-Abadi et al. (2019) studied the effect of a two-week combined supplementation of creatine, glutamine, and taurine on the response of muscle and liver damage indicators caused by high-intensity interval exercise in trained men. The levels of CK and LDH increased immediately and two hours after the selected exercise in both supplementation and placebo groups (28). Glutamine supplementation had no effect on changes in CK and LDH. Asjodi et al. (2020) compared the effect of five days of separate and simultaneous consumption of curcumin and glutamine on the indicators of muscle damage following eccentric resistance activity. A significant decrease was observed in the serum levels of CK and LDH and muscle soreness at all times following the activity in the curcumin group as well as in the curcumin and glutamine group compared to the placebo group. The glutamine group had no significant difference from the placebo group, and the curcumin and glutamine group had no significant difference with the curcumin group; thus, all the reducing effects of the serum levels of CK and LDH enzymes could be attributed to the curcumin supplement(8).

In this vein, it appears that the results of the present study are in agreement with the results of Mohajeri et al. (2021), Raizel & Tirapegui (2018), Nemati et al. (2019), Córdova-Martínez et al. (2021) and Naclario et al. (2015) (19-23) however they are inconsistent with the results of the researches of Asjodi et al. (2020), Moini Najafabadi et al. (2019), Najarzadeh et al. (2015), Rahmani-nia et al. (2012) and Coqueiro et al. (2019) (8, 10, 26-28). The difference in the results of the present study and other studies can be attributed to some factors including the type and dose as well as the processing and consumption of the glutamine used; the intensity, duration, and different types of training; differences in the age, gender, and type of subjects in terms of race and lifestyle and their conditions of health and disease as well as the difference in the type and design of human and animal model research. These findings support the effectiveness of oral glutamine supplementation in reducing muscle damage in animal models, but further studies in human samples are needed.

5. Conclusion

In conclusion, these findings demonstrate that oral supplementations with L-glutamine induce cytoprotective effects, following experimental progressive RA and attenuate harmful and injury effects of RA. Therefore, it suggests that glutamine consumption in human samples can have similar results in reducing muscle damage after progressive strength training. This property can be used to benefit from the possible protective effects of glutamine in maintaining the structure and integrity of the muscle fiber cell membrane and reducing fiber damage.

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Conflict of interests: The authors declare that there is no conflict of interest in the research.

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