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The Effect of Consume Leucine Supplement Before and After Resistance Exercise on Protein Metabolism Indices in Fasting Male Athletes

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ABSTRACT

Introduction: Fasting is one of the religious practices of Muslims during Ramadan. The aim of this study was to investigate the effect of consume Leucine supplement before and after resistance exercise on protein metabolism indices in fasting male athletes.

Methods: In this study, 33 male bodybuilders were selected and randomly divided into three groups: resistance training (N=11), resistance training and supplementation group (N=11) and control group (N=11). Subjects received 0.1 g of leucine or placebo each day. Leucine intake was 0.1 $g.kg^{-1}.d^{-1}$ of body weight. Subjects poured leucine tablets in powder form into 1 g empty capsules and dextrose as a placebo was in the form of 1 g capsules, the same shape, size and color of 1 g leucine tablets. The training protocol of the two training groups was performed for eight weeks in 3sessions per week. Each training session lasted 40 minutes. 24hour before the first training session and 24hours after the last training session, 10 ml of blood was taken from the subjects' brachial vein. To analyze the data, the statistical method of analysis of covariance was used and to determine the differences between the groups, Bonferroni post hoc test and at a significant level in all tests, $P \leq 0.05$ was considered.

Results: The findings showed that leucine supplementation before and after resistance exercise on uric acid levels in fasting male athletes was not significantly different between the two groups ($F=6.22$, $p=0.133$). However, in the amount of urea ($F=8.074$, $p=0.000$), creatinine ($F=6.106$, $p=0.001$), amount of Hypoxanthine ($F=11.511$, $p=0.002$) and Xanthine oxidase ($F=14.231$, $p=0.000$) There was a significant difference between the three groups.

Conclusion: Finally, it can be said that resistance training combined with leucine supplementation in fasting athletes can reduce protein catabolism due to exercise.

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Introduction

One of the most important goals of participating in sports activities is to promote the health of people in the community. Accordingly, maintaining the health of athletes during competitions and training has always been a topic of concern for coaches and doctors of sports teams. Recently, however, there have been concerns about some of the side effects of certain types of exercise combined with calorie restriction or weight loss on antioxidant defense systems. However, in Islamic countries, it is possible for Muslim athletes to exercise in fasting conditions (lack of access to water and food) due to the coincidence of competitions or sports camps with the holy month of Ramadan. Therefore, there is always the concern whether water and food restrictions in conditions such as Ramadan can cause a decline in performance of these athletes (1).

At rest, the energy used by the human body is predominantly derived from the oxidation of carbohydrates and fats. Blood glucose, plasma-free fatty acids, muscle glycogen, and intramuscular triglycerides are major substrate sources for energy production in skeletal muscles. The contribution of proteins to the pool of usable energy is very limited, as amino acid oxidation is usually strictly adjusted to the intake of amino acids (2).

Studies show that protein catabolism is increased in high-intensity exercise. In other words, there is a direct relationship between the intensity of exercise and the breakdown of body proteins. On the other hand, by increasing the training time below the maximum and reducing

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the body's carbohydrate reserves, the share of protein energy in exercise increases (3). Fasting with prolonged starvation (usually more than 24 hours), carbohydrate-free diet, and lowering blood glucose cause proteins to be forcibly broken down for energy and to maintain plasma glucose concentrations, and their carbon components to be used by muscles. Excretion of urea, uric acid and creatinine through urine as well as their accumulation in the blood are the reasons for the catabolism of proteins to produce energy (4).

In recent years, the improvement of adaptive responses to exercise through dietary interventions, especially the use of sports supplements, has been considered. It has also been observed that the stimulation of protein synthesis after eating certain foods is greatly influenced by the amino acid content of the diet, especially the amino acid leucine (5, 6). Leucine, isoleucine and valine are called branched chain amino acids (BCAAs). BCAAs make up about one-third of muscle protein, with leucine accounting for about 5 to 10 percent of the body's total protein. Leucine oxidation during exercise is significantly higher than isoleucine and valine. In addition, among BCAAs, leucine activates key signaling pathways for protein synthesis, such as mTOR, after exercise (7). Resistance training also stimulates protein synthesis by increasing mTOR signaling, which is a key component in regulating protein synthesis in skeletal muscle (8, 9, 10).

Regardless of age and sex, leucine resistance training and amino acid activation of mTOR leads to an increase in 4E-BP1 and S6K1 and binding of mRNA to ribosomes, thus increasing protein synthesis (11, 12).

Gil and Kim (2015) also investigated the interactive effect of leucine supplementation and resistance training on protein synthesis in rats. The results of their research showed that their perception that taking leucine with exercise could increase muscle mass was incorrect (12). New research shows that some purine derivatives, especially plasma hypoxanthine, can be considered as an indicator of intensity, and hence some limitations seen in classical indicators, such as the level of elite athletes, the level of activity intensity in training, Usability in all training courses (general, specific, competition and transfer) can be eliminated by using hypoxanthine. Hypoxanthine can be used as an indicator to estimate muscle metabolism, training level, age of athletes in various competitive and non-competitive sports, anaerobic exercise, and adaptation to their training status (13, 14, 15).

Hypoxanthine is a sign of degradation of adenine nucleotide in muscle and is an indicator of energy stress in exercise. Hypoxanthine can also be used as an indicator of the intensity and level of exercise activity (14, 15).

Fasting during Ramadan is a unique metabolic pattern in which a person abstains from eating and drinking from sunrise to sunset. It seems that changes in the number and timing of meals as well as sleep patterns during Ramadan can have different metabolic and hormonal effects. The effect of fasting on metabolism is very complex, many of the body's mechanisms work to maintain balance during the fasting period. Because in this period, in addition to physical activity, meal and bedtime are changed (16, 17) subsequently, the body's metabolism is individually altered. For this reason, it is difficult to determine the effect of each of these factors alone on metabolism during fasting.

Due to the importance of fatigue, maintaining and enhancing performance during fasting and reducing protein catabolism in athletes, using authorized sports supplements and replacing them with prohibited and illegal substances can help athletes achieve healthier as much as possible. Help achieve sports goals and success during fasting and Considering that no research was found on the effect of leucine supplementation before and after resistance activity on post-Ramadan protein breakdown indices during the fasting period of athletes, Therefore, the researcher decided to answer the question whether taking leucine supplement before and after resistance activity in fasting athletes can affect protein breakdown or not?

Material and Methods

The present study is a quasi-experimental study with pre-test and post-test design. In this study, three groups including resistance training group, resistance training and supplementation group and control group participated who have a history of regular exercise 3 times a week for one year. The subjects of the study who participated in this study voluntarily were randomly simple and equally divided into three groups and were informed of all stages of the research and the risks and possible consequences of the research

and their consent was obtained. In this study, it was tried to influence the factors and variables in the field of research and in different stages of project implementation such as nutrition, temperature, body mass index, location, age, gender, absence of diseases, status and health history, sleep schedule before Examine the test carefully. A few days before the test and before fasting, the subjects were given the necessary explanations for scheduling sleep and breakfast and iftar. Food and supplements before the test. Subjects poured leucine tablets in powder form into 1 g empty capsules and dextrose as a placebo was in the form of 1 g capsules, the same shape, size and color of 1 g leucine tablets. Leucine intake will be 0.1 g per kg of body weight. The method of use was that the number of capsules on fasting days before resistance training and after training. To standardize the test, the tests were performed at a specific time of day. Initial assessments including height, weight, body fat, BMI, and $VO2_{max}$ were performed two weeks before exercise. 24 hours before the first training session and 24 hours after the last session, urine samples were taken from the forearm of the subjects of all three groups in the fasting state (8:30 am) at a rate of 10 cc. Blood samples are centrifuged at 3000 rpm for 10 minutes and the level of the desired variables is measured with the appropriate kits purchased in the laboratory. To measure body mass index, the formula of weight to weight ratio (kg) to height (m) square two was used.

The protocol of the present study will include resistance training, each session of which consisted of three stages. The first stage was warming up for five minutes, the second stage was performing resistance training for 30 minutes. In designing resistance exercises, it was tried that the movements were multi-jointed and also included the large muscles of the lower torso, upper torso and middle limbs of the body. These exercises consisted of three sets with ten maximum repetitions, including: 1) Bench Press, 2) crunch with a bent knee, 3) leg press, 4 Back Extention, 5) Knee Flexion, 6) lateral puls, And 7) Overhead Press. In the last step, cool down for five minutes was considered. The total time of each training session was 40 minutes on average. Data collection tools and equipment include a personal information questionnaire to access the basic information of the subjects, a stadiometer made by Satrap company to measure the height of the subjects, a medical scale for measuring hand weight made in China with an accuracy of 0.01 kg to measure the weight of the subjects, Laboratory kits for measuring subjects' urine samples were (urea, uric acid and creatinine, hypoxanthine and xanthine oxidase).

In order to analyze the statistical data of this study, analysis of covariance was used to compare the mean of pre-test and post-test between groups at a significant level ($p \leq 0.05$) using SPSS 25 software.

Table 1. Mean and standard deviation of height, weight, age and BMI of subjects in groups

Groups	Age (years) M±SD	Height (cm) M±SD	Weight (kg) M±SD	BMI M±SD
Resistance training group	26.5 ± 3.12	177.1 ± 4.11	76.21 ± 4.74	24.53 ± 0.19
Resistance and supplementation training group	27.1 ± 2.81	177.6 ± 3.85	78.93 ± 4.14	25.04 ± 0.29
control group	26.7 ± 1.9	176.7 ± 3.90	76.58 ± 5.11	24.50 ± 0.54

Findings

The Kolmogorov-Smirnov test was used to determine the normal distribution of data. The results of this test showed that the data distribution was normal. For inferential analysis of data, we used parametric statistics and analysis of covariance for differences between pretest and posttest. Table 1 shows the mean and standard deviation of height, weight and age and BMI of the subjects in the groups. The results show that the distribution of subjects in both groups is almost the same.

The results of analysis of covariance did not show a significant difference between the three groups in the amount of uric acid but there was a significant difference in the levels of urea, creatinine, and hypoxanthine and xanthine oxidase between the three groups (Table 2). Bonferroni post hoc test was used for differences between groups.

According to the results of Bonferroni post hoc test, differences in the amount of all variables were observed between the resistance training group and the control group. Also, a significant difference was observed between the resistance training group and supplementation and the resistance training group (Table 3).

Table 2. Results of analysis of covariance to compare variables between groups

variable	group	Pre-test	Post-test	F	P
Uric acid content (mg / dL)	Resistance training group	4.74 ± 0.88	4.56 ± 1.07	6.22	0.133
	Resistance training and supplementation group	4.83 ± 1.41	4.69 ± 1.11		
	control group	4.48 ± 1.45	4.42 ± 1.66		
Urea level (mg / dL)	Resistance training group	40.11 ± 3.85	33.21 ± 5.44	8.074	0.000*
	Resistance training and supplementation group	36.48 ± 6.21	34.89 ± 3.98		
	control group	38.69 ± 4.74	37.25 ± 2.19		
Creatinine level (mg / dL)	Resistance training group	0.97 ± 0.12	0.76 ± 0.08	6.106	0.001*
	Resistance training and supplementation group	1.05 ± 0.09	1.02 ± 0.14		
	control group	1.11 ± 0.10	1.09 ± 0.11		
Hypoxanthine content (ng / µl)	Resistance training group	14.01 ± 2.23	16.87 ± 3.65	11.511	0.002*
	Resistance training and supplementation group	12.74 ± 3.09	13.52 ± 2.96		
	control group	11.64 ± 3.21	11.01 ± 2.11		
Xanthine oxidase content (ng / µl)	Resistance training group	25.14 ± 7.11	28.66 ± 7.23	14.231	0.000*
	Resistance training and supplementation group	27.15 ± 3.88	25.96 ± 4.47		
	control group	26.23 ± 2.89	27.24 ± 3.08		

Table 3. Results of Bonferroni post hoc test of variables in three groups

Variable	group	Resistance training group	control group
Urea (mg / dL)	Resistance training group	----------	M=10.221,P=0.000*
	Resistance training and supplementation group	M=8.63,P=0.000*	M=8.71,P=0.079
Creatinine (mg / dL)	Resistance training group	----------	M=5.095,P=0.000*
	Resistance training and supplementation group	M=5.141,P=0.000*	M=0.214,P=0123
Hypoxanthine (ng / µl)	Resistance training group	----------	M=2.857,P=0.003*
	Resistance training and supplementation group	M=4.325,P=0.006*	M=0.114,P=0.078
Xanthine oxidase (ng / µl)	Resistance training group	----------	M=5.158,P=0.009*
	Resistance training and supplementation group	M=3.325,P=0.001*	M=1.544,P=0.101

Discussion

Statistical analysis did not show a significant difference between groups in uric acid variable. Also, the results showed that resistance training significantly decreased urea, creatinine and increased hypoxanthine and xanthine oxidase. Resistance training with supplementation significantly increased urea, creatinine and significantly decreased hypoxanthine and xanthine oxidase compared to the resistance training group.

The importance of protein for athletes has long been known and the use of protein supplements in many athletes, especially strength athletes to increase their performance and performance seems to be necessary (18). The effectiveness of dietary proteins or protein supplements in athletes is such that supplementation, in addition to increasing muscle mass and preventing protein catabolism during intense or prolonged exercise, also increases glycogen synthesis after exercise and prevents sports anemia. It is associated with increased synthesis of hemoglobin, myoglobin, oxidative enzymes and mitochondria during exercise (19). Adequate protein uptake is also essential for accelerating synthesis and increasing muscle mass under these conditions. Resistance training simultaneously increases both the synthesis and breakdown of muscle proteins. But under these

conditions, muscle protein synthesis overcomes its failure, which ultimately leads to an increase in pure protein (20). Therefore, the need for protein and positive energy balance increases in those who participate in intense resistance training sessions.

There are different results about the effect of fasting on uric acid and urea. In a study, Azwany et al. examined the effect of one month of fasting on 43 Muslims. They fasted after 4 weeks, although the amount of water absorption was normal; reported a significant increase in urinary osmolality. Blood urea levels did not change significantly during 4 weeks (21). Comparing the blood samples of 19 fasting men during the first days and 23 months of Ramadan, Indral and colleagues found that serum urea, triglyceride, total cholesterol and LDL-C levels were significantly reduced (22). Azizi stated in a review study that serum uric acid levels increase abnormally during long-term starvation, possibly due to decreased glomerular filtration rate (GRF) and uric acid release. However, in Islamic fasting, there is only a slight increase in uric acid; this condition can be due to the nature of short-term and intermittent fasting. No change in uric acid may be attributed to the small number of samples studied or the high dispersion of scores (23, 24).

In the study of Bizheh et al. (2012), 12 weeks of aerobic exercise with 3 sessions per week was associated with increased aerobic capacity and decreased body mass index along with decreased uric acid (25). In this study, although aerobic exercise was used as an exercise intervention, exercise intervention led to a decrease in uric acid. This discrepancy can perhaps be attributed to weight loss and body mass index in response to aerobic exercise in the study.

The increase in urea concentration in the blood may be due to increased protein catabolism and may be due to resistance activity or decreased renal blood flow. Some studies suggest an increase in the concentration of urea in the blood, which may be due to exercise and resistance, which stimulates energy consumption and reduces energy intake. In this regard, when causing physical stress, albumin and urea excretion also increases in individuals. Exercise is one of the factors that can alter these biochemical factors. Other factors that can increase blood urea levels include increased protein in the diet, gastrointestinal bleeding and dehydration, or inadequate fluid intake, especially during fasting (26).

Shahdoost reports show similar results by examining the effect of selected aerobic exercise on protein catabolism in 15-year-old running students. However, in high-intensity training, due to the ratio of respiratory exchange, carbohydrates are the main source of energy production. Involvement of proteins in the energization of high-intensity activities also depends on training time. This means that proteins are burned more in high-intensity workouts that last longer (27). Ghanbari Niaki by studying the effect of 2 consecutive anaerobic tests with rest on female students of physical education (28), Ramezan pour by studying the effect of short-term physical activity with maximum ability on uric acid, blood urea and creatinine and urine of professional wrestlers (29), Savucu et al., By studying the effect of long-term training on blood and physical variables of adolescent female handball players (30) and Singh Bal et al., By studying the effect of plyometric training on physical and biochemical fitness parameters of high jump athletes (31)they report similar results to the present study.

The effect of aerobic activity on changes in blood urea and 24-hour urine urea with excreted urea through sweating, which indicates protein catabolism, may be due to: B) Under natural conditions and as a result of "amino acid amination", the ammonia produced is transported to the liver, where it is converted to urea by the Chris cycle during certain reactions. In exercise, this cycle also increases under active aerobic conditions and urea production (27); C) Urea produced in the body is excreted through the kidneys and urine. When glycogen stores are depleted, the muscles and kidneys use protein to provide energy during activity in the non-food environment (28).

Creatinine is produced mainly as muscle excretion and is a good measure of kidney function, because if the kidneys do not remove it from the blood, its concentration in plasma will increase. Sometimes long-term fasting, thirst, and dehydration transiently increase creatinine levels, which can be relieved by compensating for dehydration (32). Trappe et al. In the study designed a training session to evaluate the effect of exercise on net protein catabolism of excreted urea, cranitine and 3-methyl histidine. In this

study, eight healthy men rode a bicycle for 90 minutes with about 45% of their maximum oxygen consumption. During exercise, total urinary urea increased by 100% compared to before exercise, and excreted creatinine increased by 50%. Also, although the amount of excreted 3-methyl histidine tended to increase relative to creatinine, which is an indicator of protein catabolism, it did not change (33). In the present study, resistance training in the resistance training group reduced the amount of urea and creatinine, but the resistance training group with the supplementation of this reduction in urea and creatinine was less than the resistance training group, and this was probably the effect of leucine supplement on protein catabolism and it has reduced. Based on the information we have, the present study is the first to investigate the effect of leucine supplementation and fasting on protein catabolism.

Hypoxanthine is the final product in the recycling pathway of purine adenines, so that if it is converted to xanthine in the oxidation pathway by xanthine oxidase enzyme, purine is lost and finally in human it is converted back to uric acid by xanthine oxidase activity and excreted from the body. Therefore, the amount of hypoxanthine is important and can be considered as an indicator of severity (34) there is a correlation between an increase in hypoxanthine and a decrease in blood pH, and it has been shown that a critical point at 107-115% of maximum oxygen consumption is a critical point for hypoxanthine (15).

In a study by Chung Liu et al. (2005), it was reported that xanthine oxidase is the main source of free radical production in intense and tedious activities. They suggested that mitochondria play a lesser role in this type of activity. Activation of xanthine oxidase enzyme has been shown to be one of the important reasons in the production of free radicals. Xanthine oxidase is a metaflavone protein (35) that produces large amounts of free radicals by consuming oxygen, and as a result, this enzyme is one of the most important sources of O2 and H2O2 production in the body.

In the present study, resistance training in the resistance training group increased the amount of hypoxanthine and xanthine oxidase, but in the resistance training group with supplementation, the amount of hypoxanthine in the post-test increased compared to the pre-test and this increase was less than the official training group. And the amount of xanthine oxidase was observed in the resistance training group with supplementation, and this is probably the effect of leucine supplementation on protein catabolism and has reduced its amount. Based on the information we have, the present study is the first to examine the effects of leucine supplementation and fasting on hypoxanthine and xanthine oxidase levels.

Conclusion

Finally, it can be argued that resistance training combined with leucine supplementation in fasting athletes can reduce protein-induced protein catabolism. Also, it can be said that coaches and athletes can use leucine supplementation to increase performance and reduce injury to reduce catabolism caused by sports activities during Ramadan. Combining resistance training with leucine supplementation can reduce the catabolism of exercise-induced protein in fasting athletes with relative effects on urea, creatinine, hypoxanthine, and xanthine oxidase. Therefore, this solution can be considered by coaches and athletes. It seems that coaches and athletes, using knowledge-based factors affecting exercise such as nutrition, can increase performance and reduce injury.

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