



Comparison of the Effect of Leucine and L-Arginine Supplementation Before and After Resistance Training On Athletes' Protein Catabolism Indices in Ramadan

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ARTICLE INFO	ABSTRACT
<p><i>Article type:</i> Research Paper</p> <hr/> <p><i>Article History:</i> Received: 14 Jan 2022 Accepted: 23 Feb 2022 Published: 20 Mar 2022</p> <hr/> <p><i>Keywords:</i> Leucine L-Arginine Resistance training Protein catabolism Fasting</p>	<p>Introduction: Improving adaptive responses to exercise through dietary interventions, especially sports supplements, has been considered. The aim of the present study was to compare the effect of Leucine and L-arginine supplementation before and after resistance training on athletes' protein catabolism indices during Ramadan.</p> <p>Methods: In this study, 40 male bodybuilders were selected and randomly divided into four groups of resistance training (n=10) and resistance training group and Leucine supplementation (n=10), resistance training group and L-arginine supplementation (n=10) and the control group (n=10). Leucine and L-arginine tablets were poured as powder into 1g empty capsules and placebo was in the form of 1g capsules, the same shape, size and color of 1g Leucine and L-arginine tablets. The amount of supplements was 0.1g per kg of body weight. The training protocol of the two training groups was performed for eight weeks in three sessions per week. 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.</p> <p>Results: Statistical analysis did not show a significant difference between groups in uric acid variable ($p=0.097$). Also, the results showed that resistance training significantly decreased urea ($p=0.001$), creatinine ($p=0.001$) and increased hypoxanthine ($p=0.000$) and xanthine oxidase ($p=0.000$). Resistance training with Leucine and L-arginine supplementation increased there was a significant urea, creatinine and a significant decrease in hypoxanthine and xanthine oxidase compared to the resistance training group. There was a significant difference between the two groups of supplements only in the amount of xanthine oxidase. In the resistance training group and Leucine supplementation, the amount of xanthine oxidase was significantly greater than in the resistance training group and L-arginine supplementation.</p> <p>Conclusion: Finally, resistance training with Leucine and L-arginine supplementation in fasting athletes could provide a platform for reducing protein catabolism due to exercise and it can be said that coaches and athletes to reduce catabolism due to exercise. During Ramadan, they can take Leucine and L-arginine supplements to increase performance and reduce damage.</p>

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Introduction

Millions of Muslims abstain from eating and drinking for an average of 14 hours from morning to evening during the holy month of Ramadan. During Ramadan, food intake occurs more during the night, and the amount and frequency of food intake, the amount of sleep at night, and the amount of physical activity decrease (1). Different physiological responses during Ramadan are most likely the result of a disturbed sleep-wake cycle and altered eating

and drinking cycles, or most likely a combination of these factors (2). In addition, eating food at unusual times can have different metabolic effects, and lifestyle changes can have significant effects on metabolism (3).

One of the goals of sports professionals and coaches is to develop physical and mental abilities, improve sports performance and delay athletes' fatigue. Most athletes use medications and supplements to improve strength, power, speed, and endurance in order to increase

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performance and achieve success (4). Therefore, in order to gain more knowledge about the effects of taking antioxidant supplements to reduce the oxidative stress caused by intense exercise, it is necessary to conduct studies to find the best type of supplement. So far, research has been done on the effect of taking these supplements on athletes' performance and contradictory results have been obtained (5).

Studies show that protein catabolism is increased in high-intensity activities. In other words, there is a direct relationship between the intensity of exercise and the catabolism of body proteins. On the other hand, by increasing the training time below the maximum and reducing the body's carbohydrate reserves, the share of protein energy in exercise increases (6). 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 (7). Excretion of urea, uric acid and creatinine in the urine as well as their accumulation in the blood are the reasons for the catabolism of proteins to produce energy (8).

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 (9, 10). 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 rate during exercise is significantly higher than isoLeucine and valine. In addition, among BCAAs, Leucine activates key protein synthesis pathways such as mTOR after exercise (11). Resistance training also increases mTOR signaling, which is a key component in regulating protein synthesis in skeletal muscle. Stimulates protein synthesis (12). Regardless of age and sex, resistance training and the amino acid Leucine by activating mTOR increase 4E-BP1 and S6K1 and bind mRNA to ribosomes, thus increasing protein synthesis (13, 14).

Another supplement that has caught the attention of most athletes today is L-arginine, which was discovered in 1895 by Hadin et al. In mammalian protein. L-Arginine (2-amino-5-guanidinovaleric acid) is an unnecessary amino acid because it can be synthesized from the kidneys and liver (15). Under stressful conditions (severe burns, injuries, infections, etc.) it is placed in the conditional essential amino acid class. L-Arginine has many functions in the body, including protein synthesis and detoxification of ammonia waste from nitrogen catabolism (16). L-Arginine is also a precursor to the formation of nitric oxide (NO), creatine and L-glutamine. L-arginine supplementation can also increase nitric oxide levels (17). L-Arginine is found in relatively high concentrations in seafood, fruit juices, nuts, seeds, algae, meat, rice, and soy protein, and in low concentrations in the milk of many mammals (including humans, cattle, pigs). (18, 19).

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 (14). 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 intensity of activity 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 training. Hypoxanthine is a sign of degradation of adenine nucleonide 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 (20, 21).

Due to the importance of fatigue, maintaining and enhancing performance during fasting and reducing protein catabolism in athletes, the use of authorized sports supplements and replacing them with prohibited and illegal substances can help athletes achieve healthier as much as possible. Aims and help achieve sports success

during fasting, and given that a study on the comparison and effect of taking Leucine and L-arginine supplements before and after found resistance activity on protein catabolism indices during Ramadan and during fasting athletes. Therefore, the researcher decided to answer the question that taking Leucine and L-arginine supplements before and after resistance training in fasting athletes can affect protein catabolism, and which supplement will have the best effect in reducing protein catabolism?

Materials & Methods

The present study is a quasi-experimental study with pre-test and post-test design. In this study, four groups including resistance training group, resistance training group and Leucine supplementation, resistance training group and taking L-arginine supplement, 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 and equally divided into four 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. Leucine and L-arginine tablets were poured as powder into empty 1-gram capsules, and placebo was supplemented as 1-gram capsules, both in shape, size, and color, in 1-gram tablets. Consumption of Leucine and L-arginine will be 0.1 g per kg of body weight. The method of consumption 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 VO2MAX were performed two weeks before the start of training. Half an hour before the first training session and 24 hours after the last session, urine samples were taken from the forearm of the subjects in all three groups on an empty stomach (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 ratio (kg) to height (meters) to the power of two was used.

Results

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. Table 1 shows 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 four groups in the amount of uric acid, but there was a significant difference in the levels of urea, creatinine, hypoxanthine and xanthine oxidase between the four 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 all three groups. Also, a significant difference was observed between the two groups of supplements only in the amount of xanthine oxidase. In the resistance training group and Leucine supplementation, the amount of xanthine oxidase was significantly greater than in the resistance training group and L-arginine supplementation.

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

Groups	Age (years)	Height (cm)	Weight (kg)	BMI
	M±SD	M±SD	M±SD	M±SD
Resistance training group	26.5 ± 3.12	177.1 ± 4.11	76.21 ± 4.74	24.53 ± 0.19
Resistance training + Leucine supplement group	27.1 ± 2.81	177.6 ± 3.85	78.93 ± 4.14	25.04 ± 0.29
Resistance training + L-arginine supplement group	27.7 ± 3.11	178.8 ± 3.59	77.39 ± 4.36	24.18 ± 0.95
control group	26.7 ± 1.9	176.7 ± 3.90	76.58 ± 5.11	24.50 ± 0.54

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	9.885	0.097
	Resistance training +Leucine supplement group	4.83 ± 1.41	4.69 ± 1.11		
	Resistance training + L-arginine supplement group	4.59 ± 0.99	4.51 ± 1.10		
	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	10.102	0.001*
	Resistance training +Leucine supplement group	36.48 ± 6.21	34.89 ± 3.98		
	Resistance training + L-arginine supplement group	37.85 ± 5.14	35.17 ± 4.44		
	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	6.847	0.001*
	Resistance training +Leucine supplement group	1.05 ± 0.09	1.02 ± 0.14		
	Resistance training + L-arginine supplement group	1.18 ± 0.11	1.16 ± 0.08		
	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	15.474	0.000*
	Resistance training +Leucine supplement group	12.74 ± 3.09	13.52 ± 2.96		
	Resistance training + L-arginine supplement group	13.01 ± 2.21	14.12 ± 2.84		
	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	14.769	0.001*
	Resistance training +Leucine supplement group	27.15 ± 3.88	25.96 ± 4.47		
	Resistance training + L-arginine supplement group	28.21 ± 4.24	27.48 ± 5.74		
	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 + L-arginine supplement group	Resistance training +Leucine supplement group	control group
Urea (mg / dL)	Resistance training group	M=6.01,P=0.000*	M=8.63,P=0.000*	M=12.020,P=0.002*
	Resistance training + L-arginine supplement group	-----	M=9.251,P=0.124	M=8.11,P=0.085
	Resistance training + Leucine supplement group	-----	-----	M=8.71,P=0.079
Creatinine (mg / dL)	Resistance training group	M=8.23,P=0.000*	M=5.141,P=0.000*	M=10.101,P=0.000*
	Resistance training + L-arginine supplement group	-----	M=8.845,P=0.089	M=0.214,P=0.123
	Resistance training + Leucine supplement group	-----	-----	M=2.268,P=0.158
Hypoxanthine (ng / µl)	Resistance training group	M=5.44,P=0.000*	M=4.325,P=0.006*	M=3.222,P=0.000*
	Resistance training + L-arginine supplement group	-----	M=6.251,P=0.102	M=0.114,P=0.078
	Resistance training + Leucine supplement group	-----	-----	M=3.71,P=0.117
Xanthine oxidase (ng / µl)	Resistance training group	M=4.141,P=0.000*	M=3.325,P=0.001*	M=4.417,P=0.000*
	Resistance training + L-arginine supplement group	-----	M=6.058,P=0.025*	M=2.144,P=0.100
	Resistance training + Leucine supplement group	-----	-----	M=1.544,P=0.101

Discussion and Conclusion

Statistical analysis did not show a significant difference between the groups in the variable of uric acid. Also, the results showed that resistance training significantly decreased urea, creatinine and increased hypoxanthine and xanthine oxidase. Creatinine and significant reduction of hypoxanthine and xanthine oxidase were

compared to the resistance training group. There was a significant difference between the two groups of supplements only in the amount of xanthine oxidase. In the resistance training group and Leucine supplementation, the amount of xanthine oxidase was significantly greater than in the resistance training group and L-arginine supplementation.

Different physiological responses during Ramadan are most likely the result of a disturbed sleep-wake cycle and altered eating and drinking cycles, or most likely a combination of these factors (22). In addition, eating food at unusual times can have different metabolic effects, and lifestyle changes can have significant effects on metabolism (23).

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 (24). The effectiveness of dietary protein or protein supplements in athletes is such that in addition to increasing muscle mass and preventing protein catabolism during intense or prolonged exercise, it also increases glycogen synthesis after exercise and prevents anemia. Exercise is associated with increased synthesis of hemoglobin, myoglobin, oxidative enzymes and mitochondria during exercise (25). 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, the synthesis of muscle protein overcomes its breakdown, which ultimately leads to an increase in pure protein (26). 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 osmolarity. Blood urea levels did not change significantly during 4 weeks (27). Comparing the blood samples of 19 fasting men during the first days and 23 months of Ramadan, Indra and colleagues found that serum urea, triglyceride, total cholesterol and LDL-C levels were significantly reduced (28). 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 and intermittent fasting. No change in

uric acid may be attributed to the small number of samples studied or the high dispersion of scores. In the study of Bijeh 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 (29). In this study, although aerobic exercise was used as an exercise intervention, but 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 blood urea concentration 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 (30).

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 (31). In a study to evaluate the effect of exercise on net protein catabolism, Kals et al. (2000) designed an exercise session for excreted levels of urea, creatinine, and trimethyl 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 trimethylhistidine tended to increase, it did not change compared to creatinine, which is an indicator of protein catabolism (32).

In the present study, resistance training in the resistance training group reduced the amount of urea and creatinine, but the resistance training group with Leucine and L-arginine

supplementation, this reduction in urea and creatinine was less than the resistance training group, and this is probably the effect of supplements on catabolism. It is a protein and has reduced its amount. Based on the information we have, the present study is the first to compare the effects of Leucine and L-arginine supplements 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 to uric acid again 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 (33). Oxygen consumption is a critical point for hypoxanthine (34). On the other hand, hypoxanthine, because it represents ATP depletion, can be considered as an indicator of energy depletion and cellular metabolic stress (35).

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 (36) 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 (37).

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 Leucine and L-arginine supplementation, the amount of hypoxanthine increased in the post-test compared to the pre-test. There was a slight increase and the amount of xanthine oxidase in the resistance training group was observed along with taking both supplements, and this is probably the effect of the supplements on protein catabolism and has reduced its amount.

Finally, resistance training combined with Leucine and L-arginine supplementation in fasting athletes could provide a platform for reducing protein catabolism due to exercise, and it can be said that coaches and athletes to reduce catabolism due to exercise during Ramadan.

They can use Leucine and L-arginine supplements to increase performance and reduce damage. Combining resistance training with Leucine and L-arginine supplementation can reduce the protein catabolism induced by exercise in fasting athletes with relative effects on urea, creatinine, hypoxanthine and xanthine oxidase factors, so this approach can be considered by trainers. 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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