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The effect of L-Arginine supplementation on Delayed Onset Muscle Soreness (DOMS) after eccentric heavy exercise

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Original Article

Abstract

Introduction: L-Arginine supplementation improves antioxidant defenses through L-Arginine/nitric oxide pathways. This investigation assesses the influences of acuteL-Arginine supplementation on selected markers of delayed onset muscle soreness (DOMS) after one by one heavy eccentric exercise in healthy young females.

Methods: Twelve healthy young females students of the University of Mohaghegh-Ardabili voluntarily participated in a double-blind randomized-controlled crossover trial to estimate the effects of 3g L-Arginine oral supplementation versus placebo (3g glucose) following an unaccustomed heavy eccentric exercise. L-Arginine (ARG) or placebo (PLC) was taken immediately after squat exercise, separated by 10day washout. Measurements were conducted at baseline, 24, and 48h after exercise comprising: (a) TAC (b) CK (c) LDH (d) Visual Analogue Scale (VAS) (e) Range of Motion (ROM) of both knees (f) Edema of both thighs. For data analysis software SPSS version 20 was used. To analysis the data, analysis of variance with repeated measure was used.

Results: TAC significantly increased 48h after exercise compared with the pre-exercise just in ARG group ($P<0.05$). CK and LDH significantly enhanced 24, and 48h after exercise only in the PLC group ($P<0.05$). VAS and edema increased 24, and 48h after exercise compared with the pre-exercise in both groups ($P<0.05$). Moreover, ROM decreased in both groups 24, and 48h after exercise compared with the pre-exercise ($P<0.05$). There were no between-group differences in any time series ($P>0.05$).

Conclusion: The findings of this study suggest that acute supplementation of ARG after heavy eccentric exercise may alleviate muscle damage through promoting the antioxidant capacity, protein synthesis, or decrease of lactate accumulation.

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Introduction:

Unaccustomed and intense eccentric exercise is a common cause of delayed-onset muscle soreness (DOMS) (1). DOMS results in exercise performance decrease including muscle strength and range of motion (ROM) of joints for both athletes and non-athletes, and it also causes continual psychological discomfort (2,3). Beginner

and elite athletes are concerned about muscular discomfort and pain phenomena, because they can restrict further exercise and training activity (4).

The condition can lead tostop the exercise in an amateur or experienced athlete (5). The main symptoms in DOMS are muscular stiffness, tenderness, and pain during active movements (6).

There are many symptoms related to the muscle inflammation and damage such as muscle fiber swelling (7), elevated serum activities of muscle specific enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH) (8,9), as well as reduced muscle strength (10) and ROM restriction (11).

Nutritional intervention with these nutrients before and after exercise was reported to be useful in reducing DOMS. These nutritional interventions have also been reported to affect inflammatory responses and oxidative stress leading to DOMS reduction (3). Arginine is an unnecessary amino acid because the liver and kidney can synthesize it, but in conditions where the body is exposed to stresses such as infection or damage, it is a conditional essential amino acid (12).

Arginine plays a role in the synthesis of protein and detoxification of ammonia from nitrogen catabolism, as well as the pre-formation of nitric oxide (NO), L-glutamine and creatine, which has shown that arginine consumption increases levels of Nitric Oxide (13). L-arginine increases the amount of nitric oxide and reduces the production of superoxide, thereby protecting the contraction and reducing the edema after reperfusion (14). Some studies have shown that arginine is probably beneficial to the skeletal muscle that is exposed to ischemic damage through nitric oxide mediation (15).

Dietary supplements containing L-Arginine are among the most popular ergogenics intended to intensify strength, power and muscle recovery associated with both anaerobic and aerobic exercise (16). L-Arginine administration has been claimed to boost an increase in blood perfusion in the active muscle (17), increasing substrates necessary for improving muscular recovery and protein synthesis during and/or after exercise (18). L-Arginine supplementation could also improve exercise capacity by decreasing lactate accumulation, which has been shown to be involved in the development of muscular fatigue by increasing muscular acidity (19-22).

Moreover, there is an increasing interest in the role of the cellular balance between oxidative stress and antioxidant defense capacity in tissue damage and fatigue resulting from physical activity. Studies have shown that physical activityraises the production of reactive oxygen species (ROS), such as superoxide anion ($O2^{-}$). They are capable of eliciting oxidative damage to various cellular components (23,24). The extent of oxidative damage during physical activity is determined by the levels of ROS generated and the antioxidant defense capacity (25,26). When the antioxidant system is not adapted to excessive production of ROS, oxidative stress is initiated. In the recent years, a general awareness of the importance of antioxidant system in disease prevention and progression has been progressed (27).

In research, has been demonstrated the significantly effect of arginine chronic supplementation on total antioxidant capacity (28).

In our previous studies, we showed Curcumin supplementation as antioxidant is able to blunt muscle damage after one bout heavy eccentric exercise (5). We hypothesized that L-Arginine supplementation after eccentric exercise would diminish exercise-induced damage of skeletal muscle fibers in humans like rats. According to data, L-Arginine supplementation improves antioxidant defenses through L-arginine/nitric oxide pathways in exercised rats (29). To the best of our knowledge, our study is the first to investigate the effects of L-Arginine supplementation on muscle damage after a single bout of eccentric exercise in humans. Therefore, the aim of this investigation was to evaluate the effects of L-Arginine acute supplementation after an eccentric exercise on muscle damage and selected markers of DOMS.

Methods:

Subjects and supplementation

The present study was a double-blind, placebo-controlled, crossover study. Twelve healthy, young women were recruited to participate in this study (Table 1).

Table 1. Subjects characteristics

Group	(BMI)	Weight (Kg)	Height (Cm)	Age (year)	Number of subjects
A	23.42±14.1	60.53±3.23	160.6±4.91	23.5±2.6	6
B	24.05±1.23	62.30±4.58	161±4.38	23.7±2.3	6

All participants had approval from the university of Mohaghegh-Ardabiliethical advisory committee and study was based on declaration of Helsinki. All subjects were informed verbally and in writing about the nature and demands of study, and subsequently completed a health history questionnaire and gave their written informed consent. Subjects were free of antioxidant supplementation for 3 weeks prior to the study. The subjects were randomly divided into ARG supplemented group (ARG group) and a placebo group (PLC). The supplementation consistedof three capsules, each one containing 1000 mg of ARG, whereas the placebo consisted of similar capsules containing1000 mg of glucose. Capsules were administered orally immediatelyafter main trial.

In the first stage, pre-test measurements were performed before the exercise protocol and supplementation of arginine. Supplementation of arginine and placebo was done immediately after the implementation of the exercise protocol. Markers of the study were measured 24 and 48 hours after supplementation. All pre-test and post-test measurements were performed in one place (in terms of temperature, light and humidity) and time (10 am). The second stage was conducted after 10 day wash out.

Preliminary measurements

The researchers selected one repeated maximum (1RM) on the squat machine to start with a low but reasonable weight that then increased with each repetition until subjects reached their 1RM. 1RM is defined as the highest weight under which a subject is able to perform a squat position just for one repetition (5).

Experimental design and procedures

For the main trials, Squat exercise, which was used as resistance exercise to induce DOMS, was performed simply with body weight in the same manner as in the preliminary study. The exercise session consisted of seven sets of 20 squats per set (total 140 squats), with squats performed rhythmically every 2s during the sets and 3-min intervals between sets (30).

Measurement of oxidative stress, muscle damage, and DOMS markers

Blood samples were taken before exercise and supplementation, 24, and 48h after exercise. Approximately 5 ml of blood was withdrawn at each time point. Blood samples was placed in heparinized tubes and centrifuged at 3000rpm for 10min at 4°C. Plasma was transferred to microtubes and stored at -70°C for subsequent analysis. Plasma total antioxidant capacity (TAC), CK, and LDH, were measured. TAC was analyzed by Varga et al. method (31). CK and LDH were also measured by commercial available kits (Pars-Azmoon, Iran). Muscle soreness was assessed using a visual analogue pain scale (VAS), for which participants placed a mark on a 10cm line to indicate a degree of soreness. Soreness was measured by distance in centimeters from the left end of the scale to the mark. Establishment of validity and reliability of VAS as a measure for subjective soreness was cited in another report (32). ROM of the knee joints were measured by goniometer. Circumference of mid-thigh was also measured as a marker of edema around the thighs.

Dietary

Participants were instructed verbally and in writing to follow an average antioxidant diet throughout the duration of participation in the study starting 3 weeks prior to the start of the study period until the last blood sampling. A list of high antioxidant foods was provided to participants to help them avoid major sources of dietary antioxidant.

Statistical analysis

Results are expressed as means±SEM, and $P<0.05$ was consideredstatistically significant. An independent two-way analysis of variance with repeated measures was used to compare results

among treatments and over time. If a significant p value was identified for the main effect of time (time of sample), multiple pairwise comparisons were made using bonferroni confidence interval adjustment. Moreover, the dependent variable data in multiple time points between two groups were compared using independent samples t-test.

Results:

Physical characteristics of the participants were as follows (expressed as mean±standard deviation [SD]): age, 24.0±1.8 years; height, 160.1±3.1 cm; fat%, 17.2±4.2.

Total Antioxidant Capacity (TAC)

TAC showed no between group differences before and after exercise (P>0.05). TAC was significantly higher in ARG group compared with the PLC group 48h after exercise (P<0.05) (Figure 1).

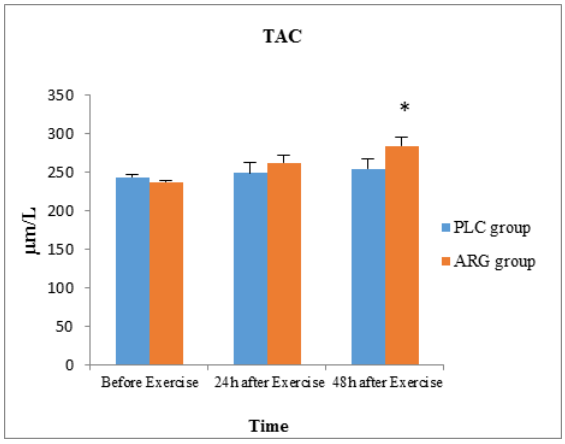

Figure 1. Plasma TAC level. Values represent means ± SEM. * demonstrates a significant increase in ARG group compared with pre-exercise (P<0.05).

CK

CK demonstrated no between groups' differences before and after exercise (P>0.05). CK significantly increased 24, and 48h after exercise in the PLC group compared with the pre-exercise (P<0.05) (Figure 2).

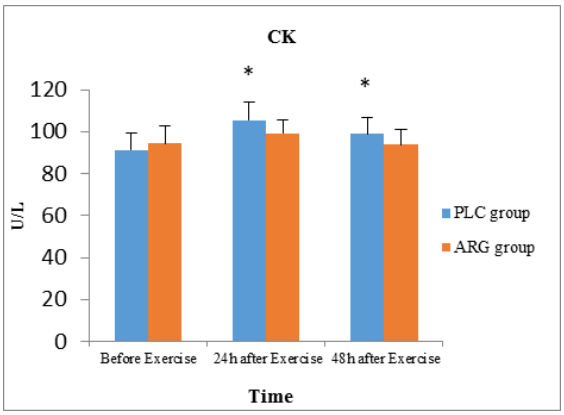

Figure 2. Plasma CKactivity. Values represent means ± SEM. * demonstrates a significant increase in PLC group compared with pre-exercise (P<0.05).

LDH

LDH demonstrated no between group differences before and after exercise (P>0.05). LDH significantly increased 24, and 48h after exercise in the PLC group compared with the pre-exercise (P<0.05) (Figure 3).

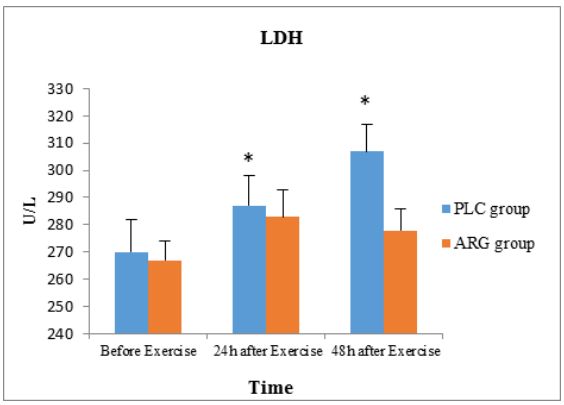

Figure 3. Plasma LDHactivity. Values represent means ± SEM. * demonstrates a significant increase in PLC group compared with pre-exercise (P<0.05).

Visual Analogues Scale (VAS)

VAS demonstrated no between group differences before and after exercise (P>0.05). VAS significantly increased 24, and 48h after exercise in both groups compared with the pre-exercise (P<0.05) (Figure 4).

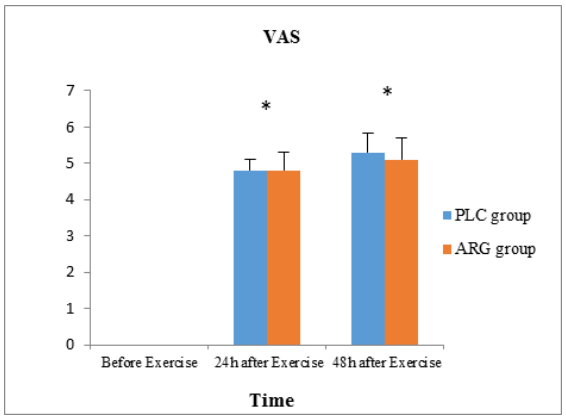

Figure 4. VAS before and after exercise. Values represent means ± SEM. * demonstrates a significant increase in both groups compared with pre-exercise (P < 0.05).

ROM mean of both Knees

ROM demonstrated no between group differences before and after exercise (P > 0.05). ROM significantly decreased 24, and 48h after exercise in both groups compared with the pre-exercise (P < 0.05) (Table 1).

Edema mean of both Thighs

Edema demonstrated no between group differences before and after exercise (P > 0.05). Edema significantly increased 24, and 48h after exercise in the both groups compared with pre-exercise (P < 0.05) (Table 2).

Table 2. ROM and Edema before and after exercise. Values represent means ± SEM. * shows a significant decrease compared with pre-exercise (P < 0.05). † demonstrates a significant increase compared with pre-exercise (P < 0.05)

	Group	Bdefore exercise	24h after exercise	48h after exercise
ROM(0)	PLC	140.20±0.53	139.40±0.60*	139.00±0.52*
	ARG	141.00±0.52	140.30±0.50*	140.10±0.55*
Edema (cm)	PLC	51.50±0.62	52.3±0.7†	53.1±0.72†
	ARG	50.90±0.57	51.88±0.57†	54.85±0.68†

Conclusion:

The main aim of this investigation was to evaluate the effect of acute L-Argenine supplementation on selected markers of DOMS.

Our study has shown that antioxidant capacity increased after acute L-Argenine supplementation and exercise. The blood level of TAC was markedly higher after exercise than pre-exercise just in the ARG group (Figure 1) that is consistent with the research findings of Fazelian et al. They assessed the effect of 3 grams of arginine supplementation on TAC in diabetic patients (28).

Research by Walker et al. examined the effect of oral administration of arginine on exercise performance and oxidative stress. Adversely, there was no consistent with the results of the present study (33). The ability of L-Argenine to promote intracellular antioxidant capacity has been reported (29). Recent findings suggested that L-Argenine supplementation can significantly enhance exercise-induced NO production and alter ROS metabolism. Indeed, there is accumulating evidence showing L-Argenine has a protective role against oxidative stress, and this action is likely mediated via its interaction with O2_ (34,35). Nevertheless, the mechanism(s) of L-Arginine supplementation's protective effects remains less understood (29).

According to our data, muscle damage enzymatic markers (CK and LDH) increased 24 and 48h after exercise compared with the pre-exercise just in PLC group (Figure 2 and 3).

CK and LDH significantly enhanced in the PLC group after exercise demonstrating occurrence of muscle damage, but in the ARG group there is no significant increase in some points of time series. There are 3 possible mechanismsmay explain the positive effects of L-Argenine on muscle damage.

The first explanation for decline of these enzymes is possible effect of L-Argenine as an antioxidant. As it can be observed in figure 3, and 4, alteration patterns of CK, and LDH is almost same as TAC. Elevation of CK and LDH plasma levels show promotion in leakage of these enzymes after exercise through cell membrane. Lipid peroxidation induced of oxidative stress may lead to

membrane permeability and the escape of muscle constituents such as CK, and LDH (36).

Therefore, the inhibitory effects of ALA on lipid peroxidation may have prevented the leakage of CK and LDH from cell membrane and consequently less increase in the CK and LDH plasma levels. Our previous studies confirmed this theory showing that antioxidant-contain supplements are able to attenuate these enzymes after heavy exercises (37-39).

The second explanation is an increase in blood perfusion in the active muscles (17). It can increase substrates necessary for improving muscular recovery and protein synthesis during and/or after exercise (18). The third possible reasoncan be improvement of exercise capacity leading to lactate accumulation decrease (22), which has been shown to be involved in the development of muscular fatigue by increasing muscular acidity (19-21).

Surprisingly, in spite of increasing of pain (VAS) and edema, and decreasing ROM of knees after exercise in the both groups, there were no within and between group significant differences after exercise (Figure 4 and Table 1).

Furthermore, our findings was contrary to recent findings regarding the effect of arginine on the reduction of muscle edema (14). It seems to reply these controversies we require more investigations in this area.

The findings of this study suggest that acute supplementation of L-Argenine after heavy eccentric exercise may alleviate muscle damage through antioxidant effects, protein synthesis, or decrease of lactate accumulation induced L-Argenine supplementation.

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