

## The Effect of Glutamine Supplementation on Delayed Onset Muscle Soreness and Skin Temperature in Untrained Elderly Male People with Type 2 Diabetes

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### Abstract

**Objective:** Effect of resistance training on muscle mass increase, blood glucose control, hemoglobin (HbA1c) reduction, on type 2 diabetes (T2DM), has been approved. While injuries and delayed onset muscle soreness (DOMS) may lead to some difficulties in diabetic patients to continue training exercise.

The purpose of this study was to investigate the effect of glutamine supplementation (0.1 g / 1kg / each day for 4 weeks) on DOMS, creatine kinase (CK) contraction and the skin temperature of the elbow flexing muscles in the untrained T2DM patients.

**Materials and Methods:** The research was an experimental study. This study was a double blinded randomized controlled trial. Subjects were randomly assigned into 4 groups of diabetic glutamine (N=10), diabetic placebo of Maltodextrin (N=10), healthy glutamine (N=10) and healthy placebo of Maltodextrin (N=10). Variables were measured before, 24, 48 and 72 Hours after exercise. Data were analyzed by ANOVA and Bonferroni's post hoc test in SPSS-18 software.

**Results:** Glutamine was effective on changes in CK and pain sensation 72 hours after exercise in diabetics. (*P*-value: 0.0001). In skin temperature was not significant different.

**Conclusion:** Glutamine was not effective in preventing and reducing DOMS, but it may reduce the DOMS period.

**Keywords:** Type II diabetes, Glutamine, Muscle soreness, Skin temperature

### Introduction

Diabetes mellitus (DM) is prevalent in developing and developed countries (1). Some studies have approved the impacts of resistance trainings in control of the blood glucose and hemoglobin A1c (HbA1c) (2,3). Some studies showed that the

combination of aerobic and resistance trainings has a significant effect on the glycemic control, muscular strength and insulin sensitivity (4). The resistance trainings are necessary part of any special training program for diabetic patients. The

improvement of insulin sensitivity in these people is correlated with increased muscle mass resulting from resistance trainings (5,6). Resistance trainings might have greater advantages for diabetic people, for example, they can enhance the inactive muscle fibers, enhance the function of muscles and increase the muscular mass (7,8).

However, resistance training program in people who were not active physically for a long time can induce delayed onset muscle soreness (DOMS). This group of people also might lose their willingness to continue training (9). DOMS is a phenomenon, which happens due to impact of unusual trainings in skeletal muscle. The pain will not be felt up to about 10 hours after training, it is known as DOMS (10). The pain caused by DOMS can vary from mild to severe pain. The pain and discomfort caused by DOMS will reach to its peak 24 to 48 hours after training, and it might last for seven days (11).

Some studies showed that DOMS caused by eccentric contractions, while may any type of intense and unusual training lead to DOMS (10-12). One of the main introduced mechanisms is the inflammation in the muscles and increase of blood flow. Thus, the onset of inflammatory processes in the damaged muscle increases the blood flow, leading to increased skin temperature in the related muscle (13,14).

Diabetic patients do not experience pain correctly because of diabetes neuropathy. So, the non-invasive method of measuring the skin temperature can be more useful in such patients. Reliability and validity of thermic image to measure the status of painful muscles have been approved recently (15). The DOMS in diabetic patients might be related to the free radicals and reduction of the proteins synthesis. These changes might prolong the improvement process. As diabetic people suffer from neuropathic disorder, they might prone to more damages due to lack of correct feeling of soreness and early returning to training (16).

Glutamine supplement is using to prevent or reduce DOMS. It seems that glutamate of skeletal muscle and other tissues plays regulatory role in the proteins synthesis of all parts of body (17,18). Reduction in glutamine stores may reduce the protective function of glutamine against apoptosis initiating in neutrophils, macrophages and lymphocytes (19).

Glutamine is the precursor of glutathione. By increasing the antioxidant capacity of the plasma, glutathione decreases the lipid peroxidation and free radicals. Moreover, glutamine can leave its anti-inflammatory impact by decreasing the formation of prostaglandins (20-22). Some of studies have also reported the impact of glutamine supplement on reduction of insulin resistance (23-25).

DOMS is one of the most common damage caused by training, which lead to negative impact on the people function (26). Much research has been carried out on delayed onset soreness in young people, but there is little information on the delayed onset soreness among the diabetic people and elderly people. In addition, the impact of glutamine supplementation on muscle damages in diabetic patients is not studied.

The current research aims to answer to these questions:

- 1- Is there any difference between these patients and healthy people in recognizing the DOMS?
- 2- Does taking glutamine supplementation have impact on DOMS in diabetic people and healthy people?

## Materials and Methods

Studied sample were selected among Ghazvin Diabetes Association and Ghazvin Medical-Sports Board patients. Twenty studied sample were selected among type 2 diabetes mellitus (T2DM). In addition, 20 non-trained males were selected among non-diabetic healthy candidates. Subjects were selected based on convenient sampling. The subjects and the examiners were blinded about intervention,

and the subjects were randomly allocated into either the experiment group, or the placebo group.

T2DM patients were informed of their disease for less than 10 years, had no diabetes neuropathy, and without any other disease affecting the neuropathic status. In addition, 20 non-trained males were selected among non-diabetic healthy candidates. The age range of subjects was  $65 \pm 2.7$  years. In past 6 months had no supplement and they did not participate in resistance training program. Subjects were informed about the study and they signed the written consent.

Subjects were excluded if they have hepatic diseases, rhabdomyolysis, or an impaired circulatory disease (such as Raynaud's), any recent upper limb injuries, hypertension, high doses of alpha or beta Agonist/antagonists, cox 2 inhibitors, calcium channel blockers, or pregabalins. Also, subjects were advised not to take any pain reducers, NSAID, or dietary supplements during the course of the study. The subjects were obliged not to take anti-inflammatory drugs or other supplements during various stages of research and their diet was controlled in terms of amount of taking the food containing glutamine and vitamin C. The Subjects with past history of cardiovascular, renal disease, hepatic disease, surgical procedure and smoking were excluded. Then, subjects were randomly divided into four groups (each group containing 10 subjects). The first group included the diabetic people taking glutamine. The second group included diabetic people taking the placebo of Maltodextrin. The third group included the healthy people taking glutamine, and the fourth group included healthy people taking the placebo of Maltodextrin

### Training protocol

The maximum dynamic strength of the subjects was obtained in elbow flexion movement with one repetition maximum test and 70% of maximum strength of subjects was calculated. They performed front of arm

movement with dumbbell in 5 sets with maximum repetition. The subjects performed the trainings while their elbow was on the thigh. The rest time between two sets was considered to be 90 seconds.

### Supplementation

In the current research, the glutamine supplementation and the placebo of Maltodextrin, manufactured by Pooyan Nutrition Company (PNC) were selected. The consumption amount per kg of body weight was calculated 0.1 g for each of the subjects and it was provided for subjects in same packaging. Subjects consumed placebo and glutamine in combination with 300 ml of water for a period of 3 days per week (after launch) for 4 weeks.

### Diet control of subjects

Subjects were asked to complete the nutritional note to investigate the food habits of each subject and to approve their eligibility to participate in the study. Diet was assessed at baseline using the Food Frequency Questionnaire (FFQ) (17). Daily nutritional notes were evaluated by food analysis software 2.5.3, which has been updated for Iranian foods. It was proven that the subjects have consumed the glutamine and placebo, prescribed for them.

### Measurement of the plasma creatine kinase enzyme concentration

Serum level of creatine kinase enzyme was measured by an auto-analyzer device (Hitachi, manufactured by Japan) and by CK-NAC (EC2.7.3.2) kit manufactured by Pars Azmoon Company and by using IFCC/DGKC method.

### Measurement of skin temperature using Infra-Red camera (Optris PI 160)

A thermic camera (Optris PI 160) was used in the current research. The considered images were taken from arm of the subjects at a distance of one meter and temperature of  $23^{\circ}$  C. In addition, special software of thermic Infra-Red camera Optris Pi Conect

(Rel.2.7.2132.0) was used in this research. Using this software, the mean and SD temperature of the considered areas were recorded from  $-20^{\circ}\text{C}$  to  $+100^{\circ}\text{C}$  (Figure 1).

### Measuring soreness response the using McGill pain questionnaire

McGill pain questionnaire was used in the current research to measure the soreness response of subjects. This questionnaire validity to assess the feeling pain of subjects has been approved by some researchers (26). The pain feeling of subjects was assessed in this study at four stages of before training and 24, 48, and 72 hours after training.

### Statistical analysis

The present research was conducted under the supervision of the Ethics Committee of Shahid Babaiee University of Medical Sciences at IR.QUMS.REC.1396.291. Means and standard errors of mean (SEM) were calculated. Measurements of all variables (Creatine kinase, SF-MPQ, and Skin Temperatures) were compared overtime between the experimental and placebo groups of each of

the 2 main groups (healthy and diabetic). Mauchly's sphericity test was a statistical test used to validate a repeated measures analysis of variance (ANOVA) to confirm the equality of the variance of all combinations, the spindle test was used and the m-box test was used to examine the covariance between the groups. Data were analyzed for time and group intervariability using repeated measures analysis of variances. When significant difference over time was found using Bonferroni post hoc test between group's comparisons for subject characteristics were done using student-Newman-Keuls test and the level of Significant was set at  $P < 0.05$ .

### Results

The baseline characteristics of studied patients were presented in table 1. Our findings indicated the significant increases in the creatine kinase level after training in all groups ( $P$ -value: 0.01). Findings of Bonferoni post hoc test suggested that resistance training affected the changes in creatine kinase enzyme 24, 48, and 72 hours after training ( $P$ -value: 0.001). The mean creatine kinase in the third

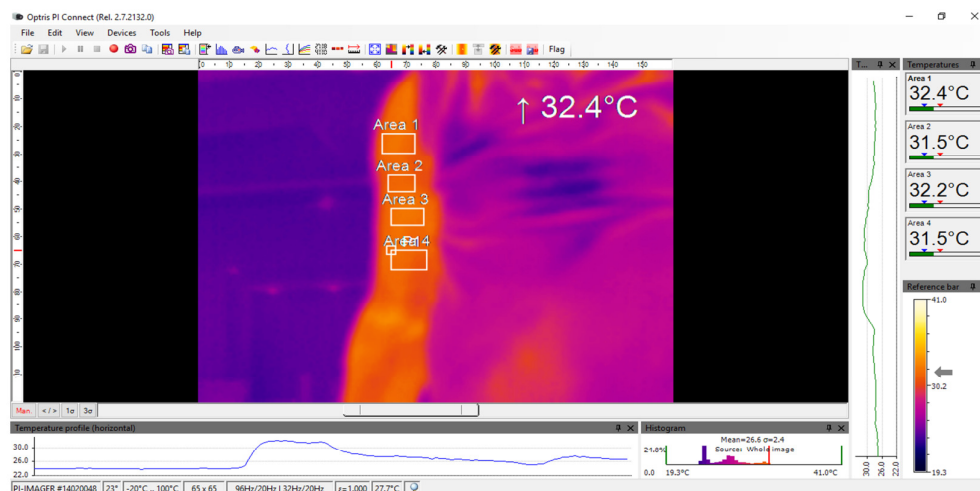


Figure 1. View of special software of Infra-Red camera Optris Pi Conect (Rel.2.7.2132.0)

Table1. Means ( $\pm$  standard error of mean) of the baseline characteristics

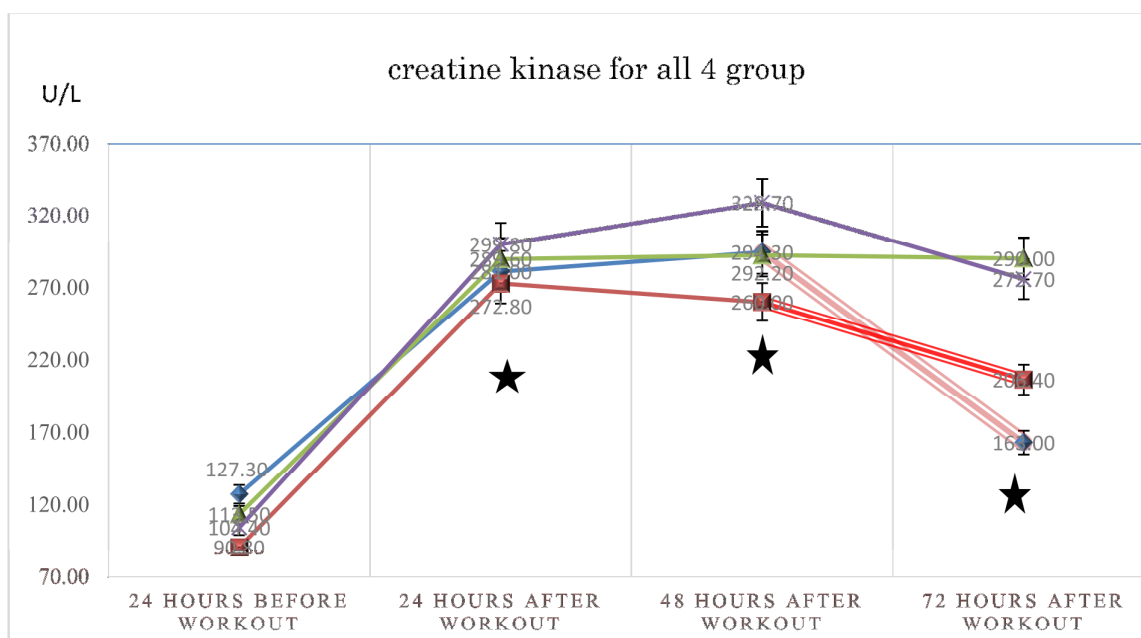
Subject type	N	Age (Years)	Height (cm)	Weight (kg)	BMI (Kg/m <sup>2</sup> )	HbA1c (%)
Diabetic individuals	20	64.42 ( $\pm 2.7$ )	171.34 ( $\pm 9.2$ )	85.56 ( $\pm 14.8$ )	28.54 ( $\pm 9.2$ )	7.3 ( $\pm 1.7$ )
Healthy individuals	20	63.76 ( $\pm 3.6$ )	167.91 ( $\pm 14.5$ )	79.09 ( $\pm 20.6$ )	29.32 ( $\pm 8.2$ )	5.37 ( $\pm 1.02$ )
P-value	-	0.301	1.000	0.932	0.435	-

day showed reduction in all groups (figure 2), but high level of it was found in diabetic group who took the placebo. No significant difference was observed in the inter-group creatine kinase increase. However, complementary investigation revealed that the level of creatine kinase 72 hours after training in the experimental diabetic group was significantly less than that in other groups, especially in comparison to the control diabetic control ( $P$ -value: 0.001). This suggested that glutamine is effective in faster return of creatine kinase to the base level. The highest creatine kinase level was also seen in diabetic people took the placebo.

Figure 3 illustrates that skin temperature after training increases 48 hours after training, while it followed decreasing trend in the third day (72 hours after training), except for diabetic people took the placebo) and this increase occurred with slight slope after the first day. Findings indicated significant difference between skin temperatures in the flexure muscles of elbow in different days. Findings of Bonferoni post hoc test ( $P$ -value: 0.001) indicate a significant difference

between skin temperatures before training and 48, 24, and 72 hours after the training. Hence, the skin temperature can be used as one of the syndromes of delayed soreness. While skin temperature in the control diabetic group is higher after the training compared to that in experimental group, findings showed that taking glutamine has no impact on the changes in skin temperature ( $P$ -value: 0.266).

Figure 4 illustrates that the mean of feeling pain followed an increasing trend up to 72 hours after training in all groups, except for diabetic people who took glutamine. The diabetic people who took glutamine were faced with decreasing feeling pain 48 years after training. The feeling pain in the healthy people took glutamine, as other groups, followed an increasing trend, but its level is less than that in two placebo healthy groups ( $P$ -value: 0.001). Findings suggest that feeling pain before training has significant difference with other days. Thus, training was effective in feeling pain. The inter-groups findings suggested that taking glutamine had significant impact on the feeling the pain level in different groups. Difference in the level of feeling pain



**Figure 2.** Graph of plasma creatine kinase enzyme concentrations (Means  $\pm$ SEM) in the healthy group who consumed the glutamine supplement (squares), the healthy group who consumed the placebo supplement (X), the diabetic group who consumed the glutamine supplement (diamonds), and the diabetic group who consumed the placebo supplement (triangles)



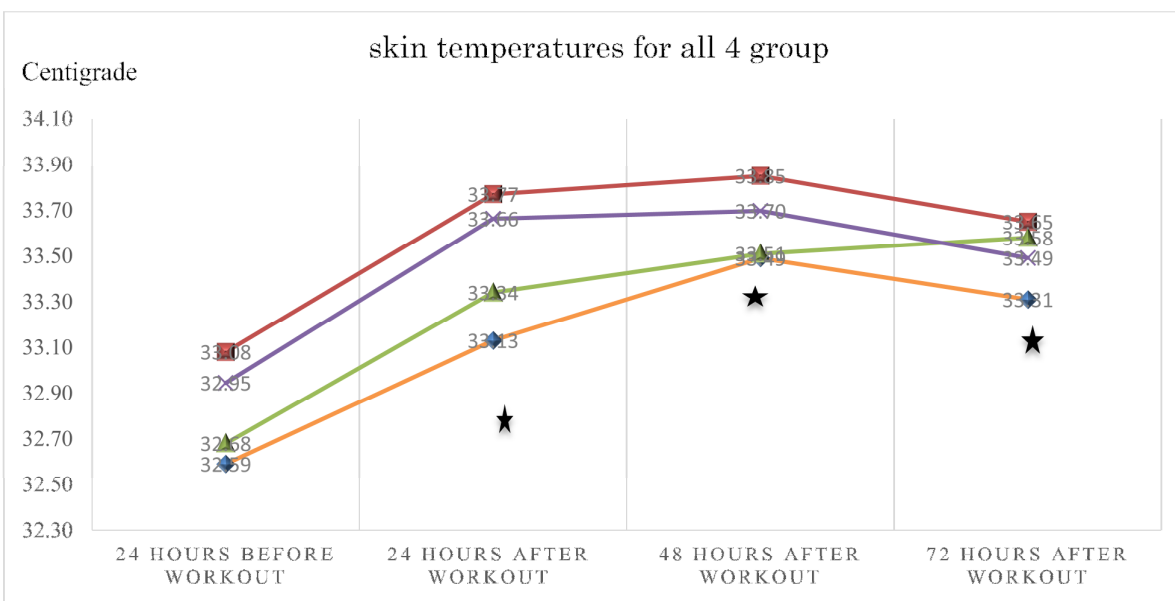


Figure 3. Graph of skin temperature (Means  $\pm$ SEM) in the healthy group who consumed the glutamine supplement (squares), the healthy group who consumed the placebo supplement (X), the diabetic group who consumed the glutamine supplement (diamonds), and the diabetic group who consumed the placebo supplement (triangles).

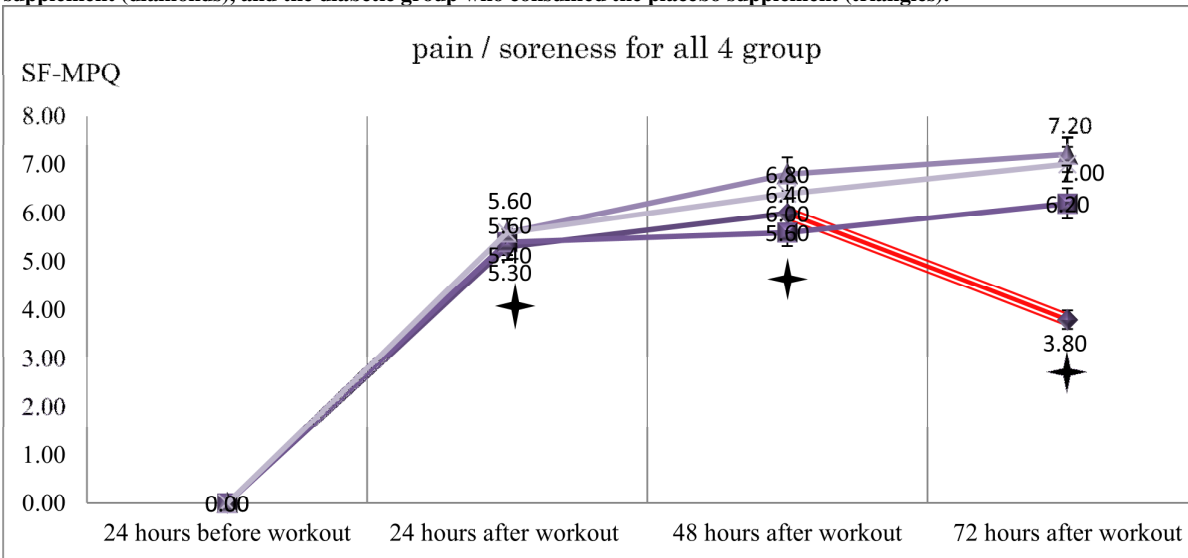


Figure 4. Graph of the soreness responses using the SF-MPQ (Means  $\pm$ SEM) in the healthy group who consumed the glutamine supplement (squares), the healthy group who consumed the placebo supplement (X), the diabetic group who consumed the glutamine supplement (diamonds), and the diabetic group who consumed the placebo supplement (triangles).

was seen in only 72 hours after training between the mean of pain feeling in diabetic people took the glutamine and other groups ( $P$ -value: 0.001) and no significant difference was seen among the other groups.

## Discussion

The aim of this research was evaluating the impact of taking glutamine supplement on DOMS, including creatine kinase enzyme, skin temperature, and the level of feeling the pain in untrained male people (non-active) suffering from T2DM. The level of CPK

increases in people suffering from progressive muscle atrophy and other neuromuscular diseases. In addition, it has been found that DOMS caused by unusual physical activities, especially in eccentric contractions, increases the activity of certain enzymes such as LDH and CPK (11). Some researchers have reported that taking the glutamine is effective in indicators of DOMS (28,25). However, Rahmani Nia, et al stated that glutamine does not have significant impact on creatine kinase (17). Other findings of the current research suggest that while there are differences between creatine kinase enzyme changes in diabetic people and healthy people, these differences cannot be considered significant. Additionally, the impact of glutamine on changes in creatine kinase enzyme is not significant 24 and 48 hours after training. While the level of creatine kinase in the glutamine diabetic group was significantly higher than that of other groups 72 hours after resistance training, especially in the placebo diabetic group, the impact of taking glutamine after 72 hours might be related to the fact that glutamine supplement in diabetics can reduce the period of improvement in the delayed onset soreness.

One of the syndromes of DOMS considered in this research is skin temperature of the biceps muscle. Other studies used thermic camera to measure muscle soreness as a result of resistance trainings (15,29). In general, skin temperature changes after training up to 48 hours followed an increasing trend in the mentioned research, while it followed decreasing trend 72 hours after training (except for diabetic people took the placebo), and this increase was with slight slope after the first day. No significant difference was found between skin temperature changes in diabetic people and healthy people, and the impact of taking the supplement on the level of skin temperature was not significant. Findings revealed that changes in the skin temperature could be regarded as a valid index in measurement of delayed onset soreness, and this new technique can be considered in

assessing the muscular damages by using a thermic camera, since findings of the current research are in line with findings of the similar studies in terms of changes in skin temperature changes along with significant changes found in other syndromes like creatine kinase enzyme, feeling pain, change in range of movement, and muscle strength. Significant increase in skin temperature resulting from resistance training is in line with findings reported by Nakhil and Petrovsky. (15,29)

This research revealed that the pattern of changes in the skin temperature in diabetic people took the supplement is different from that of those who took placebo, while this difference was not significant. Hence, taking this dose of glutamine supplement has no impact on changes in skin temperature. Other syndrome investigated in the current research was level of feeling pain. Researchers reported that damage in muscle or connective tissue will result in an increase in the number of blood neutrophils. Neutrophils are transferred to damaged area, leading into an increase in the number of monocytes. Along with maximum feeling of muscle pain, the number of monocytes reaches to its peak (48 hours after training). Monocytes cause production of great number of prostaglandins. It also stimulates the neural terminals in the muscles and the feeling of muscle soreness is created as a result (11). In general, feeling of pain in this research followed an increasing trend (except for diabetic group took glutamine) and this increase was with slight slope after the first day. However, diabetic people taking glutamate were faced with reduced feeling of pain 48 hours after training. Research findings revealed no significant difference in feeling the pain among the diabetic people and healthy people. Researchers reported that diabetic people with at least neuropathy disorder score of 1, as healthy people, would experience delayed onset soreness caused by unusual trainings (15,29). However, inconsistent findings might be obtained in diabetic people with more severe neuropathy and in patients suffering from metabolic and endothelial

disorders, so further studies are required in this regard. The current research revealed that diabetic people taking glutamine had the lowest level of feeling pain, while the control diabetic people experienced the highest level of feeling pain, and significant difference was found between these two groups in terms of feeling pain 72 hours after training. This difference can be attributed to impact of glutamine in inhibiting the prostaglandins production, stimulating the neural terminals of neural fibers or the impact of glutamine in increasing the capacity of protein synthesis and antioxidant capacity of glutamine (20,21,23). It is recommended complementary studies to be carried out to measure the syndromes related to inflammatory factors and synthesis of protein.

## Conclusions

Significant changes were found in plasma creatine kinase, skin temperature in the biceps muscle area, feeling pain 24, 48, and 72 hours after training, compared to baseline. It

suggests the impact of training on development of DOMS in this study. However, the levels of creatine kinase and feeling pain in the diabetic group and healthy group took glutamine supplement were greater than those of control group only 72 hours after training. It suggests that taking glutamine had no impact on preventing and reducing the DOMS, while it was effective in reducing the period of DOMS. Taking or non-taking a drug or a supplement cannot be recommended by relying on findings of clinical trials, so it is recommended that other studies to be carried out in order to examine the impact of other doses of glutamine on level of DOMS among the diabetic people.

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