Influence of glutamine supplementation on muscle damage and oxidative stress indices following 14km running

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Original Article

Abstract

Introduction: The aim of this study was to evaluate effects of 7 day glutamine supplementation on exercise-induced oxidative stress and muscle damage.

Methods: Nineteen healthy, nonsmoking, young men were recruited to participate in this study. Participants were randomized in a double-blind placebo-controlled fashion into 2 groups: Glutamine (G group) (n=9) and placebo (P group) (n=10). Subjects consumed daily either glutamine (1.5 g/kg glutamine+250ml water+15g sweetener) or placebo (250ml water+15g sweetener) for 7days. Then, participants ran 14km and were allowed to consume water throughout the exercise. Blood samples were taken before supplementation, before exercise, immediately, and 1h after exercise.

Results: Creatine kinase (CK) significantly increased in both groups after exercise compared with pre-exercise ($P<0.05$). Moreover, there was a significant CK increase in P group compared with the G group 1h after exercise ($P=0.021$). TAC significantly increased immediately post-exercise compared with pre-exercise just in the G group ($P<0.05$). There was significant reduced glutathione (GSH) increase in the G group after supplementation, immediately, and 1h after exercise, but just 1h after exercise in P group compared with baseline ($P<0.05$). Malondealdehyde (MDA) significantly increased 1h after exercise compared with pre-exercise just in P group ($P<0.05$). There was any within or between groups' differences in serum total bilirubin ($P>0.05$).

Conclusion: It seems 7 day glutamine supplementation has been able to affect oxidative stress and muscle damage markers via possibly an effect on antioxidant agents.

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Introduction:

The cells in our body permanently produce free radicals and reactive oxygen species (ROS) as part of metabolic processes (1). Oxidative stress can occur as a consequence of a general increase in ROS generation, a depression of the antioxidant defense system (enzymatic and non-enzymatic), or both (2). Oxidative stress causes damage to biologic macromolecules such as nucleic acids, membrane lipids, and proteins, and hence disrupts normal physiological function (3). Evidence for increased

ROS production during and following exercise is provided by numerous investigations that have noted an increase in various oxidative stress biomarkers following both acute aerobic and anaerobic exercise (4,5). Although regular exercise are associated with reduced risks of all-cause mortality (6), strenuous exercises because of an insufficiency of endogenous antioxidants may cause antioxidant defense systems to be temporarily overwhelmed. Thus, supplementation of these systems with antioxidants may reduce oxidative stress (7). Measurement of various antioxidant or oxidant parameters can be used to determine the risk of oxidative stress or the effectiveness of antioxidant supplementation (8).

Mireover, strenuous exercise increase blood level of some enzymes such as CK and LDH demonstrating the damage of muscle (9-11).

Muscle tissue may be damaged following intense prolonged training as a consequence of both metabolic and mechanical factors. Indeed, rhabdomyolysis may result from direct and indirect damage to the muscle membrane, and may lead to leakage of intracellular muscle components such as CK and LDH into the extracellular fluid owing to possibly damages of skeletal muscle cell structure at the level of sarcolemma and Z-disks (12,13).

Glutamine is an important constituent of proteins and is a precursor for the synthesis of amino acids, nucleotides, nucleic acids, amino sugars, and several other biologically important molecules (14).

Glutamine, a nonessential amino acid, has received increasing attention because it becomes essential during stress and catabolic conditions (15).

Its administration can result in an enhanced antioxidant capacity in various situations, such as critical illness or sepsis (16). According to Antonio et al., there are no side-effects following 6day supplementation of 1-3g per kilogram glutamine (17). Moreover, Gleeson et al. showed that acute intake of 20-30g glutamine seem to be without ill effect in healthy adult humans and no harm was reported in which athletes consumed 29-8g glutamine everyday for 14 days (18). It seems glutamine exerts its antioxidant property through promotion of reduced glutathione synthesis (16). For example, Sasaki et al. showed 3g glutamine supplementation for two weeks, decrease muscle damage and oxidative stress in Judoists. According to their data, Glutamine supplementation has prevented excessive muscle damage and suppression of neutrophil function, especially in ROS production activity, even during an intensive training period (19). However, Rahmani-Nia et al. reported no significant decrease in muscle damage (CK), 24 and 48 after resistance exercise in spite of 4week glutamine supplementation (20).

Strenuous physical exercise as well as prolonged endurance-like programs lead to glutamine depletion due to lowered synthesis and enhanced uptake by liver and immune cells (21). On the other hand, prophylactic and therapeutic nutritional interventions involving protein, protein hydrolysate, mixed amino acids, selective amino acids, and branched-chain amino acids have been demonstrated to be effective in reducing some or all of the symptoms of muscle damage following endurance exercise (22,23).

To the best of our knowledge, there is a paucity of investigations as well as controversy about the prophylactic effects of glutamine supplementation on exercise-induced oxidative stress and muscle damage. Therefore, the aim of this study is to evaluate influence of one week glutamine supplementation on selected markers of muscle damage and oxidative stress after 14 km running.

Methods:

This study is a double-blind, placebo-controlled study. Of all 30 volunteer male students of Islamic Azad University, Ardabil branch, 19 healthy, nonsmoking, young men were randomly recruited to participate in this study. The protocol of the study was approved by the university ethics committee in accordance with the Helsinki Declaration. All participants 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. They were eligible to participate to the study if they: a) had not a history of medical or surgical procedures that might significantly affect the study outcome, including cardiovascular disease or metabolic, renal, hepatic, or musculoskeletal disorders; b) were not smokers or used medication that might have significantly affected the study outcome; c) not used any nutritional supplements

(i.e. creatine, protein drinks, amino acids, vitamins) in the 8 weeks before the beginning of the study; d) had not enrolled in another trial or ingested another experimental product in the 30 days before screening and enrollment and e) having VO_2max between 40-50 ml/kg/min. Physical characteristics as well as running record of subjects has been shown in table1. There were any significant differences in these characteristics in both groups.

Experimental design

All procedures were completed at laboratory of Islamic Azad University, Ardabil branch. Two weeks prior to main test, participants underwent Cooper test for determining their VO_2max (24). Then, participants were randomized in a double-blind, placebo-controlled fashion into two groups: Glutamine (G group) (n=9), and placebo (P group) (n=10). They arrived at laboratory after an overnight fasting. A baseline blood draw was taken. Then, subjects consumed daily either placebo (250ml water+15g sweetener) or the glutamine supplement (1.5g/kg glutamine+250ml water+15g sweetener) for 7 days. Afterwards, on the day of the test, subjects attended at athletic arena after an overnight fasting. After second blood taking, participants were allowed to have a standard breakfast consisting of bread and jam. Two and a half hour later, participants warmed up for 15min consisting of running at 50%VO_2max (10min) and stretching (5min). Then, Participants ran 14km. In our previous works, we have observed that 14km running in healthy young men would lead oxidative stress (5). Participants were allowed to consume water ad libitum throughout the trial. Blood samples were taken immediately and 1h after exercise.

Blood sampling and analysis

Approximately 10ml of blood was withdrawn at each time point. Three milliliters of blood was placed in heparinized tubes and centrifuged at 4000 rpm for 10min. Plasma was transferred to microtubes and stored at -80°C for subsequent analysis. The rest of the blood was allowed to clot and centrifuged at 4000rpm for 20min. Serum was removed and aliquoted in 0.2ml volumes and stored at -80°C until analysis. Serum creatine kinase (CK) and bilirubin were measured using commercial available kits (Darmankav, Iran) using Spectrophotometer (Covergent, England). Total antioxidant capacity (TAC) was analyzed by Varga et al. method (25). We used the FRAP assay, which depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH. (Fe (II)-TPTZ has an intensive blue color and can be monitored at 593nm.

Reduced glutathione (GSH) was measured using Ellman method (26). In this method we determined total sulfhydryl groups, protein-bound sulfhydryl groups, and free sulfhydryl groups in biological samples using DTNB. Finally, Malondialdehyde (MDA) was analyzed using a spectrophotometric method (27). In this method, Samples was homogenized with aqueous trichloroacetic acid in the presence of hexane and butylated hydroxytoluene, and the homogenate was centrifuged. Following reaction with thiobarbituric acid reagent, malondialdehyde was directly quantified on the basis of the third-derivative absorption spectrum of the pink complex formed.

Statistical analysis

Results are expressed as mean±standard error. Data were analyzed for time and group inter-variability using two way repeated measures analysis of variance (Two-way ANOVA). When appropriate, significant differences among means were tested using Bonferroni post hoc test. Between groups comparison for subject characteristics was done using unpaired t-test. Differences between groups were considered to be significant when $P<0.05$.

Results:

There were no significant differences between physical characteristics of both groups ($P>0.05$) (Table 1).

Antioxidant Marker

TAC increased immediately after exercise compared with pre-exercise just in G group ($P<0.05$). There were no significant differences between groups (Figure1).

Oxidative Stress Markers

GSH increased immediately after supplementation, immediately, and 1h after exercise compared with baseline in G group ($P<0.05$).

There was significant increase in P group just 1h after exercise compared with baseline ($P<0.05$) (Figure 2).

Table 1. Physical characteristics of subjects in Placebo (P) and Glutamine (G) groups. Values for each group represent means±SEM

Groups	Age (year)	Stature (cm)	Body mass (kg)	VO_{2max} ($ml.kg^{-1}.min^{-1}$)	Running records (min)
P (n=10)	22.40±0.97	177.8±2.21	74.60±3.32	44.71±1.83	83.40±5.49
G (n=9)	24.55±0.80	182.3±3.22	82.56±2.63	44.07±1.75	86.67±4.90

Table 2. Bilirubin before and after exercise. There are no within and between groups' differences. Values represent means ± SEM (n=9)

Markers	Groups	Baseline	Pre-exercise	Post-exercise	1h after exercise
Bilirubin	P (n=10)	0.43±0.05	0.31±0.5	0.42±0.03	0.30±0.03
	G (n=9)	0.41±0.05	0.37±0.04	0.43±0.07	0.30±0.06

Muscle injury markers

CK significantly increased immediately, and 1h after exercise in both groups, and 1h after exercise just in P group compared with pre-exercise ($P<0.05$) There was significant increase 1h after exercise in P group compared with G group ($P=0.021$) (Figure 4). Bilirubin showed no significant within and between groups differences ($P>0.05$) (Table 2).

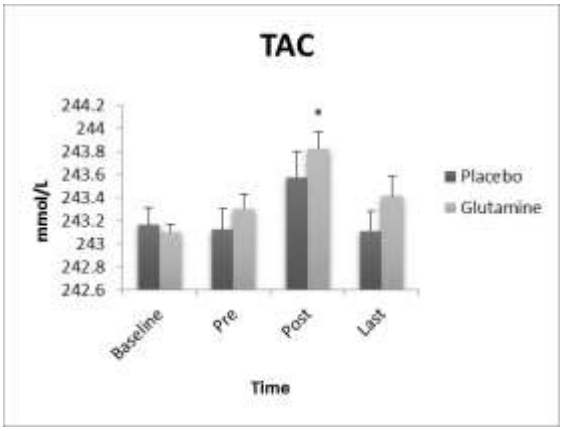

Figure 1. TAC before and after exercise. * Values represent within group significant increase compared with pre-exercise in G groups ($P<0.05$). Values represent means±SEM

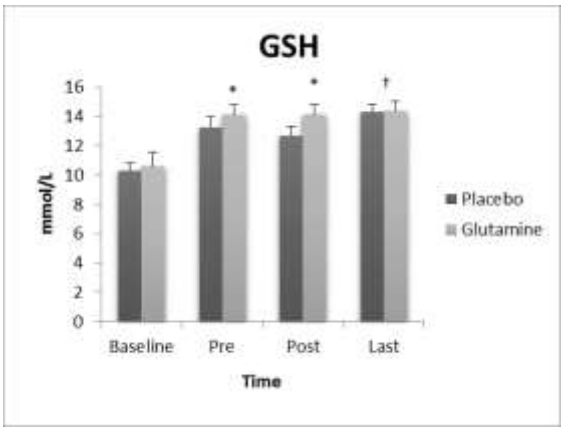

Figure 2. GSH before and after exercise. * Values represent significant increase in G group compared with Baseline ($P<0.05$). †Values represent significant increase compared with baseline in both groups ($P<0.05$). Values represent means±SEM

MDA significantly increased 1h after exercise compared with pre-exercise just in P group ($P<0.05$) (Figure 3).

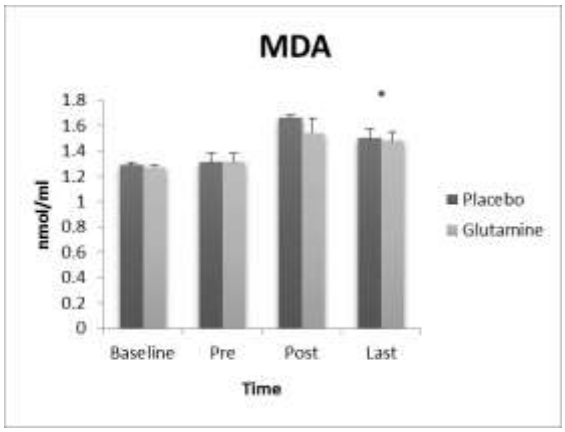

Figure 3. MDA-TBARS before and after exercise. * Values represent significant increase just in P group compared with pre-exercise (P < 0.05). Values represent means ± SEM

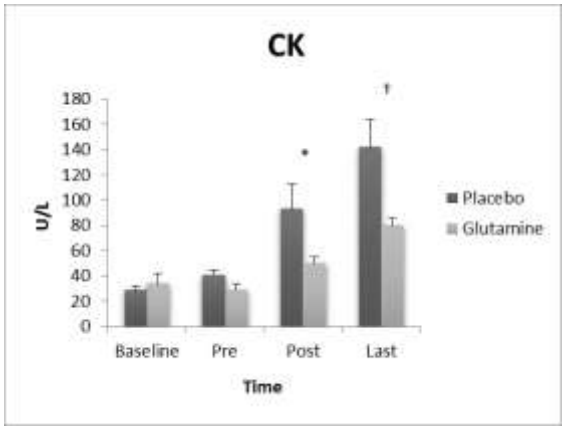

Figure 4. CK before and after exercise. * Values represent significant increase in G group compared with Baseline (P < 0.05). † Values represent significant increase in P group compared with baseline and G group (P < 0.05). Values represent means ± SEM

Conclusion:

The purpose of this study was to evaluate effect of 7 day glutamine supplementation on exercise-induced oxidative stress and muscle damage markers.

Increase in TAC following exercise was reported (11,28). Our study demonstrates an increase in plasma TAC level immediately after exercise just in G group, showing possible effect of glutamine supplementation as an antioxidant. The reason of enhancement of TAC in G group may be due to increase of GSH contents of cells after supplementation and exercise (Figure 2).

Decrease in GSH level after exercise as a marker of oxidative stress has been reported (28). Our study showed significant increase in plasma GSH after supplementation, immediately, and 1h after exercise in G group, but just 1h after exercise in P group, demonstrating the GSH synthesis promotion from glutamine sources (16). Similar to present study, in our previous investigation, chronic supplementation with MSM as an antioxidant was able to prevent a further decrease in plasma GSH level (29).

Current study also presents the MDA level of plasma as a marker of lipid peroxidation. The increase of lipid peroxidation following 14km running was confirmed in our previous works (2,24). Opposite our results, the majority of studies which utilized specific measure of MDA have noted no increase in MDA following exercise (10).

Current study showed significant increase just in P group 1h after exercise compared with pre-exercise, demonstrating the possible effect of glutamine supplementation as an antioxidant. The possible explanation for no within-group enhancement of MDA in G group compared with P group is possibly because of promotion of GSH contents and antioxidant capacity following glutamine supplementation.

According our data, 14km running has been able to elevate CK immediately, and 1h after exercise compared with pre-exercise in both groups. Moreover, there was significant decline in G group CK serum compared with P group 1h after exercise indicating possible influence of supplementation on CK Our results were in agreement with Sasaki et al. and Ionescu et al. (19,30) and opposite to Rahmani-Nia et al. (20).

There are some possible explanations for the effect of glutamine supplementation on serum CK concentration:

The first is the probable effect of glutamine as an antioxidant. Elevation of CK serum concentration shows promotion in leakage of these enzymes after exercise through cell membrane. Cell membrane damage may be resulted from lipid peroxidation. Lipid peroxidation is one of the most common occurrences during oxidative stress. Glutamine as an antioxidant potentially manages to scavenge free radicals and decrease oxidative stress and lipid peroxidation. According to some

researches, lipid peroxidation may lead to membrane permeability and the escape of muscle constituents such as CK (31,32). Inhibitory effects of glutamine on lipid peroxidation may have prevented leakage of CK from cell membrane and consequently alleviation of CK serum. The result of this study is in agreement with our previous studies showing the effect of antioxidants supplementation on CK as an indirect marker of muscle damage (10,11,33,34). The other mechanisms by which glutamine supplementation may attenuate symptoms of muscle damage are: greater amino acid availability (35,36), extra energy intake from supplementation (35), increased protein synthesis and/or decreased protein breakdown producing a positive net protein balance (15,37). For example, Waddell et al. showed administering an L-glutamine supplement to mice through their drinking water, their skeletal muscle contractile forces were increased. With a greater contractile force, they conclude that the mice receiving the L-glutamine experienced greater muscle growth. Large muscles produce greater forces of contraction due to the increased number of myofilaments available to create a muscle contraction (38).

Bilirubin is one of the other markers of muscle damage. Some studies reported the elevation of bilirubin after exercise (39). In present study, total bilirubin serum level showed no difference before and after exercise in both groups opposite to our previous investigations (29). Bilirubin level seems to depend on the oxidative stress and the oxidative stress can induce heme oxygenase and leads to heme degradation and bilirubin synthesis (40). It seems glutamine supplementation has not been able to affect this pass way, but the reason is unclear for us.

It seems the controversy in the influence of glutamine supplementation on exercise-induced oxidative stress and muscle damage is due to discrepancy in kind of exercise, dose and duration of supplementation. Therefore, for clarifying the effect of glutamine, it is required more studies in this area.

There were some limitations in current study should be considered for future investigations. In this study, glutamine consentration in blood did not measured. Furthermore, MDA and GSH was mot measured by golden standard method.

The present study suggests acute strenuous bout of exercise could lead oxidative stress on healthy young men. One week supplementation of glutamine has some alleviating effects on lipid peroxidation and may augment intracellular antioxidant system and prevent increase in CK following acute exercise. Nevertheless, the exact mechanism of glutamine on attenuating the markers of oxidative stress and muscle damage is not well established and further exploration is needed.

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تأثیر مصرف مکمل گلوتامین بر شاخص‌های آسیب عضلانی و استرس اکسیداتیو متعاقب ۱۴ کیلومتر دویدن

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مجله پزشکی هرمزگان سال بیستم شماره پنجم ۹۵ صفحات ۳۳۱-۳۲۳

چکیده

مقدمه: *هدف از مطالعه حاضر، ارزیابی تأثیر مصرف ۷ روز مکمل گلوتامین بر استرس اکسیداتیو و آسیب عضلانی ناشی از فعالیت بدنی می‌باشد.*

روش کار: *۱۹ مرد جوان سالم و غیرسیگاری در این مطالعه شرکت کردند. آزمودنی‌ها در یک مدل دو سویه کور با گروه کنترل به دو گروه تقسیم شدند: گروه گلوتامین (۹ نفر) و گروه دارونما (۱۰ نفر). آزمودنی ها به مدت هفت روز، روزانه ۱/۵ میلی‌گرم در کیلوگرم گلوتامین به اضافه ۲۵۰ میلی‌لیتر آب و ۱۵ گرم شیرین کننده در گروه گلوتامین و یا ۲۵۰ میلی‌لیتر آب به اضافه ۱۵ گرم شیرین کننده در گروه دارونما مصرف کردند و سپس ۱۴ کیلومتر دویدند. آزمودنی‌ها در تمام طول فعالیت، به دلخواه مجاز به مصرف آب بودند. نمونه های خونی قبل از مصرف مکمل، قبل از فعالیت، بلافاصله و یک ساعت پس از فعالیت اخذ گردید.*

نتایج: *کراتین کیناز (CK) به طور معنی‌داری در هر دو گروه پس از فعالیت نسبت به قبل از آن افزایش یافت ($P<0/05$). همچنین، کاهش معنی‌داری در گروه گلوتامین در ارتباط با CK نسبت به گروه دارونما ملاحظه گردید ($P<0/021$). ظرفیت آنتی‌اکسیدانی تام (TAC) در گروه گلوتامین پس از فعالیت نسبت به قلب از آن افزایش معنی‌داری نشان داد ($P<0/021$). گلوتاتیون احیا شده (GSH) در گروه گلوتامین، پس از مصرف مکمل، بلافاصله و یک ساعت پس از فعالیت افزایش معنی‌دار نشان داد، در حالی که در گروه دارونما فقط یک ساعت پس از فعالیت افزایش معنی‌دار مشاهده شد ($P<0/05$). مالون دی آلدئید (MDA)، فقط در گروه دارونما نسبت به قبل از فعالیت افزایش معنی دار نشان داد ($P<0/05$). در ارتباط با بیلی روبین، هیچ‌گونه تغییر درون گروهی و بین گروهی ملاحظه نشد ($P>0/05$).*

نتیجه‌گیری: *به نظر می‌رسد ۷ روز مصرف مکمل گلوتامین توانسته است از طریق خاصیت آنتی‌اکسیدانی بر استرس اکسیداتیو و آسیب عضلانی ناشی از فعالیت تأثیر بگذارد.*

کلیدواژه‌ها: *گلوتامین، آسیب عضلانی، کراتین کیناز*

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