

The effects of creatine and carnitine supplementation on oxidative stress and inflammation in athletes

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Original Article

Abstract

Introduction: The present study was carried out to evaluate the effects of creatine and carnitine supplementation on oxidative stress and inflammation in athletes.

Methods: In this single-blind randomized clinical trial 45 sportsmen were studied selected from Velayat Sports Complex (in Shiraz) in this single-blind randomized clinical trial. Then they were randomly divided into four groups supplemented with: a) Creatine, b) Carnitine, c) Creatine and Carnitine and d) placebo for 28 days. Before and after the intervention, blood samples were taken for measurement of oxidative stress (MDA and GSHPx) and inflammation markers. Data were analyzed using SPSS/PC statistical package (version 18). Paired t-test and ANOVA were used to compare mean values of each groups.

Results: No statistically significant difference were found between the IL-6 and MDA mean levels of the intervention groups and placebo group ($P > 0.05$). Furthermore, no statistically significant difference was noted in GSHPx mean values before and after supplementation as well as in comparison to placebo group.

Conclusion: Based on the defined dose and duration of supplementation in the present study, creatine and carnitine supplementation showed no effects on oxidative stress and inflammation conditions either separately or simultaneously. Furthermore, no negative and side effects were observed.

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Introduction:

Nowadays, nutritional supplements taken by athletes is a common practice aiming at enhancement of athletic performance, work-out improvement, better recovery and health (1,2). Consumption of ergogenic substances is observed

in professional, beginner athletes and even in non-athletes community. Several studies report high prevalence of ergogenic substances consumption in Iran. Seventy four to 91 percent of athletes use nutritional supplements (1-4). There are currently more than 600 nutritional supplements including creatine and carnitine in the market (5). Creatine

with 400 million dollars sale per annum is one of the most popular supplements (4-7).

Carnitine (L-3-hydroxy trimethyl amino butanoate) is a nutrient and similar to vitamins. Small quantities of carnitine are synthesized endogenously from two essential amino acids, lysine and methionine in liver and kidneys (8). Athletes use it as an ergogenic substance for endurance activities (9,10).

Sport increases production of Reactive Oxygen Species (ROS) resulting from higher Oxygen consumption, temperature rise in muscles mitochondria, and tissue damages (11). In addition to pre-inflammatory activities, ROS can cause production of Lipid Hydroperoxides (LH) by peroxidization of lipids. The LH; in turn, are disintegrated to some Aldehydes like Malondialdehyde (MDA). They then react with nucleophile active loci in DNA, proteins and phospholipids resulting in various damages (11).

Plasma levels of inflammatory proteins including Interleukin 6 (IL-6) increase during and after exercise. The levels are associated with muscular damages (12). Some studies recently reported the anti-oxidative and anti-inflammatory effects of creatine and carnitine (12,13). Löster and Böhm (2001) reported that intracardiac injection of carnitine after ischemic reperfusion in mice reduced the level of Malondialdehyde (MDA) (14). Rahimi (2011) also reported reduction of MDA with daily 20 g supplementation of creatine for 7 days in comparison with a placebo group (15). Akbarari et al, (2009) indicate that 1 g carnitine supplementation for 6 weeks did not increase IL-6 post exercise level in 19 male swimmer students (13). Bassit et al (2007) also reported that supplementation of 20 g daily for 5 days inhibits increase of IL-6 level after a sport tournament (12).

Glutathione Peroxidase (GSHPx) - an enzyme found in mitochondria, cytosol and cell membrane - reconstructs and neutralizes hydrogen peroxide (H_2O_2) and organic hydro peroxidases use reduced glutathione (GSH) as electron donor. It is also known as one of the oxidative stress indices (16). There are few researches studying the effect of creatine or carnitine supplementation on the level of GSHPx in athletes. They have reported

different results. For example, Basta et al (2006) reported that supplementation of 20 g creatine for 5 days followed by 10 g creatine for 30 days in 20 sportsmen reduced the level of Malondialdehyde and GSHPx insignificantly. The authors associated it to antioxidant effects of creatine (17).

This study aims to evaluate the effects of creatine and carnitine supplementation on oxidative stress and inflammation in athletes because: 1) there are limited studies with controversial results, 2) no studies have investigated the effects of simultaneous use of creatine and carnitine on inflammatory indices and oxidative stress in athletes, and 3) whether simultaneous use of creatine and carnitine preferred to single use or not?.

Methods:

Sampling and study design:

This single-blinded randomized clinical trial study was carried out with a before and after of intervention design. Fifty four martial athletes from Velayat Sport Complex (in Shiraz) volunteered to participate in the study. Out of that number 48 athletes were selected to participate the study including 25 Karate and 23 Taekwondo athletes. Inclusion criteria were: the age of 18 to 30, at least 1 month experience in the current martial athletic practice, lack of using protein supplements within the past 6 months before study and no history of liver, kidney, heart, pulmonary and musculoskeletal diseases. All the martial athletes completed an informed consent form following which their height and weight were measured by a body weighing scale equipped with stadiometer. The data were obtained using questionnaire investigated in a face-to-face way. The subjects were randomly divided into four groups of 12 members. After collecting blood samples supplements and placebo distributed among the subjects daily for 28 days. At the end of the course, blood samples were taken again.

Supplementation and exercise protocol:

Supplementation was as follow: 12 martial athletes on creatine supplement (Group 1) received 0.3 g for each kilogram weight for 7 days (loading dose) followed by 0.03 g for each kilogram of weight for 21 days (maintenance dose) (18); 12 martial athletes on carnitine supplement (Group 2) received 2 g carnitine for 28 days (19); 12 martial athletes simultaneously on creatine and carnitine supplements (Group 3) received creatine as Group 1 and carnitine as per Group 2; and 12 martial athletes in Group 4 received 8 g placebo daily for 28 days.

Creatine (monohydrate creatine 98%, crystallized powder) and carnitine (powder) – both made in Germany (Sigma) purchased and Placebo (mixture of Cellulose and Avicel) were prepared. The group members were provided with supplements in identical packs. They were asked to take the supplements four times a day (fasting, am, pm and in the evening) along with fruit juice.

Throughout the study course, the athletes continued their routine exercise schedule (3 sessions of 2 hours per week, totally 12 exercise sessions). The sessions were monitored and reported by the supervisor of the study plan.

Measurement of blood parameters:

To measure the parameters (Malondialdehyde, GSHPx and IL-6), 5 ml blood (12 to 14 hours fasting) was taken from athletes' brachial veins in a medical laboratory. To prepare serum, the samples were centrifuged 4000 rpm for 10 minutes. One day prior to sampling, the athletes were not allowed to have sport activities/exercise.

MDA, glutathione peroxidase were measured by spectrophotometer device with colorimetric and ELISA methods; respectively, using commercial

kits of Pars Azmun Company. IL-6 was measured with IRA method by Gamma counter device.

Statistical Methods:

Data were analyzed using SPSS statistical software package (version 16). Mean and standard deviation were used to describe, and Paired t-test was used to compare the variables before and after intervention in each group. Moreover, to compare the mean of variables between the groups, ANOVA was used. Confidence interval for all the tests was considered 95%.

Results:

Out of 48 athletes participating in the study, three individuals were excluded from the study: 2 withdrew from the study and 1 left due to foot fracture. The study ended with 45 subjects. Table 1 shows the demographic information of the study subjects.

Indices for oxidative stress and inflammation for the group taking creatine and the group consuming carnitine, for the group taking creatine-carnitine and the group consuming placebo are respectively in table 2 and table 3.

Comparison of indices for oxidative stress and inflammation in groups supplemented with creatine, carnitine, creatine-carnitine and the group taking placebo before and after intervention were not significant ($P > 0.05$).

Comparison of the indices for oxidative stress and inflammation between the groups using ANOVA after completion of supplementation course revealed no significant difference between the groups ($P > 0.05$).

Table 1. Demographic of the athletes participating in the study by the groups of the study

Group	Members	Age (Years)	Weight (kg)	Height (cm)	BMI (kg/m ²)
Cratine	12	22±1.2	65±9.1	1.76±0.09	21±1.4
Carnitine	12	21±1.1	67±9.2	1.77±0.08	21±2
Cratine-Carnitine	11	22±1.4	73±9.9	1.76±0.02	23±3.1
Placebo	10	22±2.4	74±9.3	1.76±0.07	23±2

Values are based on mean ± standard deviation (M ± SD)

Table 2. Comparison of indices for oxidative stress and inflammation in the group taking creatine and the group taking carnitine before and after intervention

Variable	Crative taking Creatine			Group taking Carnitine		
	Before	After	P-value*	Before	After	P-value*
IL-6 [†]	15.4±1.7	14.9±1.6	0.49	17.5±3.9	15.7±1.9	0.10
MDA ^{††}	3.3±0.9	3.1±1	0.18	3.3±0.7	3±1.2	0.13
GSHPx ^{†††}	50±7	52±8	0.07	51±2	52±3	0.25

[†] Pg/ml ^{††} μ mol ^{†††} ng/ml

* denotes that the difference between the values after and before intervention are significant (Paired t-test, P<0.05); mean±standard deviation (M±SD)

Table 3. Comparison of stress oxidative and inflammation in cratine-carnitine and placebo receiving group before and after intervention

Variable	Group taking Creatine-Carnitine			Group taking Placebo		
	Before	After	P-value*	Before	After	P-value*
IL-6 [†]	15.7±1.8	14.8±2.1	0.34	16.4±1.5	14.7±2	0.12
MDA ^{††}	3.31±1	3±1.2	0.13	3.3±0.8	3.2±0.9	0.20
GSHPx ^{†††}	51±2	52±2	0.33	47±5	48±5	0.42

[†] Pg/ml ^{††} μ mol ^{†††} ng/ml

* denotes that the difference between the values after and before intervention are significant (Paired t-test, P<0.05); mean± standard deviation (M±SD)

Conclusion:

Since there were few studies with rather limited and controversial studies on the effect of short-term and long-term creatine and carnitine supplementation on factors involving inflammation and oxidative stress in athletes, patients and even animal, the present study evaluated the effects of long-term creatine and carnitine on inflammatory and oxidative factors in athletes.

The present study did not find any significant comparison of the mean levels of IL-6 in any of the groups before and after supplementation by creatine, carnitine, creatine-carnitine and placebo. Although mean of IL-6 serum level in all the groups decreased after the intervention, the reduction was not significant in any of the groups. However, some studies reported carnitine supplementation reduced or prevented the increase of IL-6. Akbari et al reported that carnitine supplementation prevented the increase of IL-6 concentration (13). Winter et al also reported that carnitine supplementation reduced inflammatory cytokines including IL-6 in mice suffering from

cancer (8). In these studies, it was noted in the study that carnitine reduced muscular cell damage probably by improving cell membrane stability which resulted in reduction of inflammatory proteins in athletes and patients (13).

A study by Bassit et al on the effects of creatine on the levels of IL-6 revealed that creatine supplementation did not reduce the levels of IL-6; however, it prevented its increase (12). Jafari et al in a short-term creatine monohydrate supplementation study also reported that the increase of inflammatory factors (IL-6) levels after doing exercise comparing with the group receiving placebo was significantly less (19,20). In the two cited studies, it was noted that 1) increase of IL-6 for 24 hours after doing exercise was probably necessary to maintain the glucose levels, and 2) creatine monohydrate reduced the levels of IL-6 by (a) increasing muscular glycogen content, (b) increasing glutathione content followed by reduction of hydrogen peroxidase and inhibition of NfKB (12,20). But in our study, no significant reduction was observed with supplementation of creatine, carnitine and even creatine-carnitine. The

reason for the reduction reported in the earlier studies may be associated with course duration, severity, the course of sport exercise (11), dose, supplementation course and nutrition status of the participating in the studies. Since nutrition is one of the major and effective factors influencing inflammatory indices including IL-6 (21), ignoring the status of nutrition in the study can be considered one the limitations of the present study as well as other studies. And the mentioned effect for creatine and carnitine supplementation can be associated to this case.

In the present study, the concentration of MDA reduced insignificantly in all the groups after intervention. The findings are not consistent with some other studies. For example, Löster & Böhm indicated that carnitine reduced production of MDA in cardiac cells of mice with cardiac ischemia (14). A clear mechanism for the effect of carnitine supplementation on MDA levels has not been specified and the published findings on the mechanism of carnitine in reduction of lipid peroxidation are contradictory. It can be concluded that carnitine cannot act as a free radical scavenging, because it should also affect in low doses for the exertion of antioxidant effects (14, 22, 23).

Other studies have reported contradictory results in this regard. Rahimi in his study of short-term effects of creatine on athletes reported that creatine supplementation reduced plasma MDA insignificantly (15). This study associated the reduction of MDA to indirect antioxidant effects of creatine including presence of Arginine in the structure of creatine and production of NO through that process, and presence of Sulfur bearing components (cysteine and methionine) in the structure of creatine which are both sensitive to free radicals. Although Basta reported insignificant reduction of MDA with creatine supplementation (17), his results are consistent with the current study.

There are few independent studies about the effects of creatine or carnitine supplementation on glutathione peroxidase in athletes. The present study showed insignificant increase of glutathione peroxidase levels after intervention. The results of some studies are consistent with our results. For example, Rani et al reported improvement of

glutathione status in the brain of old mice supplemented with carnitine. They believed that it was due to protective effects of carnitine on Vitamins E, C and glutathione reductase resulting in insignificant increase of glutathione peroxidase (24). But the results of the present study are different from Basta's study who reported significant reduction of glutathione peroxidase by creatine supplementation. Basta attributed the significant reduction of glutathione peroxidase to indirect antioxidant effects of creatine (17). Some studies indicated that regular sports result in the increase of plasma glutathione peroxidase (16). Hence, it can be concluded that the insignificant increase reported in some studies including the present study may be associated to sport and not to creatine or carnitine supplementation.

Overall, according to the findings of the present study, although long-term creatine and carnitine separately or simultaneously - using the protocol explained in this study - did not have positive effects on oxidative stress and inflammation in athletes, it did not have undesirable complications as well. It can be concluded that creatine-carnitine simultaneous supplementation is not remarkably different from single supplementation of either creatine or cotinine to affect on oxidative and inflammatory factors. Martial sports coaches and martial athletes can be assured that separate or simultaneous creatine and carnitine supplementation have neither positive effects on indices for inflammatory and oxidative stress nor undesirable complications on the indices. If they like to take the supplements, they should use them cautiously and after consulting with a specialist. Moreover, considering the lack of studies in this field, further researches in the field are necessary.

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اثرات مکمل یاری کراتین و کارنیتین بر شاخص‌های استرس اکسیداتیو و التهاب در ورزشکاران

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چکیده

مقدمه: مطالعه حاضر با هدف بررسی اثرات مکمل یاری همزمان کراتین - کارنیتین بر استرس اکسیداتیو و التهاب در ورزشکاران انجام شد.

روش کار: در این کارآزمایی بالینی تصادفی شده یک سوکور، تعداد ۴۵ ورزشکار مرد از مجموعه ورزشی ولایت شهر شیراز انتخاب شده و به طور تصادفی به چهار گروه دریافت‌کننده: الف) کراتین، ب) کارنیتین، ج) کراتین-کارنیتین و د) دارونما تقسیم شده و به مدت ۲۸ روز مکمل یاری شدند. قبل از شروع مکمل یاری و بعد از اتمام دوره مداخله نمونه خون جهت اندازه‌گیری شاخص‌های استرس اکسیداتیو (مالون دی آلدئید و گلووتاتیون پراکسیداز) و التهاب (ایترلوکین ۶) از ورزشکاران جمع‌آوری شد. داده‌ها با استفاده از نرم‌افزار آماری SPSS تحلیل شدند. برای مقایسه متغیرهای مورد مطالعه قبل و بعد از مداخله در هر گروه از آزمون زوجها و برای مقایسه میانگین متغیرها میان چند گروه از آنالیز واریانس استفاده شد.

نتایج: ایترلوکین ۶ و مالون دی آلدئید پس از اتمام دوره در هیچ یک از گروهها تغییر معنی‌داری نسبت به پیش از آن و نسبت به گروه دارونما نشان نداد، گلووتاتیون پراکسیداز همچنین پس از اتمام دوره در همه گروهها نسبت به قبل و نسبت به گروه دارونما تغییر معنی‌داری را نشان نداد.

نتیجه‌گیری: بر اساس دوز مصرفی و مدت مکمل یاری در این مطالعه، اگرچه مکمل یاری کراتین و کارنیتین به صورت جداگانه و همزمان اثرات مثبتی بر استرس اکسیداتیو و التهاب نشان نداد، اما اثرات منفی و عوارض جانبی نیز مشاهده نشد.

کلیدواژه‌ها: کراتین - کارنیتین - استرس اکسیداتیو - التهاب

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