

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

Zohreh Mazloom<sup>1</sup> Bahman Panahandeh<sup>2</sup> Mohsen Salesi<sup>3</sup> Mohammad Hassan Eftekhari<sup>1</sup>

Associate Professor Department of Nutrition<sup>1</sup>, Professor Department of Physical Education<sup>3</sup>, Shiraz University, Shiraz, Iran. PhD Student Department of Nutrition<sup>2</sup>, Tehran University of Medical Sciences, Tehran, Iran. Assistant

(Received 22 Dec, 2012)

Accepted 30 Dec, 2013)

## 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.

*Correspondence:*

Bahman Panahandeh,  
PhD Student,  
School of Nutritional Sciences  
and Dietetics, Tehran  
University of Medical  
Sciences,  
Tehran, Iran  
Tel: +98 9171439363  
Email:  
Panahandeh.b@hotmail.com

**Key words:** Creatine – Carnitine - Oxidative Stress - Inflammation

**Citation:** Mazloom Z, Panahandeh B, Salesi M, Eftekhari M.H. The effects of creatine and carnitine supplementation on oxidative stress and inflammation in athletes. *Hormozgan Medical Journal* 2014;18(5):375-381.

### 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 <sup>2</sup> ) |
|-------------------|---------|-------------|-------------|-------------|--------------------------|
| 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 <sup>†</sup>    | 15.4±1.7                | 14.9±1.6 | 0.49     | 17.5±3.9               | 15.7±1.9 | 0.10     |
| MDA <sup>††</sup>    | 3.3±0.9                 | 3.1±1    | 0.18     | 3.3±0.7                | 3±1.2    | 0.13     |
| GSHPx <sup>†††</sup> | 50±7                    | 52±8     | 0.07     | 51±2                   | 52±3     | 0.25     |

<sup>†</sup> Pg/ml    <sup>††</sup>  $\mu$ mol    <sup>†††</sup> 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 <sup>†</sup>    | 15.7±1.8                        | 14.8±2.1 | 0.34     | 16.4±1.5             | 14.7±2  | 0.12     |
| MDA <sup>††</sup>    | 3.31±1                          | 3±1.2    | 0.13     | 3.3±0.8              | 3.2±0.9 | 0.20     |
| GSHPx <sup>†††</sup> | 51±2                            | 52±2     | 0.33     | 47±5                 | 48±5    | 0.42     |

<sup>†</sup> Pg/ml    <sup>††</sup>  $\mu$ mol    <sup>†††</sup> 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.

#### **Acknowledgement:**

This article was adopted from a Master of Science thesis conducted by Mr. Bahman Panahandeh. Authors would like to express their gratitude the Vice-chancellor for Research at Shiraz University of Medical Sciences. Moreover, we would like to thank all the athletes taking part in the study for their cooperation.

## References:

1. McDowall JA. Supplement use by young athletes. *J Sports Sci Med.* 2007;6:337-342.
2. Maughan RJ. Nutritional ergogenic aids and exercise performance. *Nutr Res Rev.* 1999;12:255-280.
3. Kargarfard M, Kashi A, Sarlak Z. Prevalence of drug use and knowledge of the effects and side effects of doping in athletes province of lorestan. *The Scientific Journal of Mental Health.* 2009;2:123-134. [Persian]
4. Hoffman J, Ratamess N, Kang J, Manqine G, Faiqen baum A, Stout J. Effect of Creatine and  $\beta$ -Alanine Supplementation on Performance and Endocrine Responses in Strength/Power Athletes. *Int J Sport Nutr Exerc Metab.* 2006;16:430-446.
5. Gaeini AA. Nutritional approaches in exercise & weight control. 5<sup>th</sup> ed. Tehran: Bamdad Press; 2011. [Persian]
6. Gotshalk LA, Kraemer WJ, Mendonca MA, Vingren JL, Kenny AM, Spiering BA, et al. Creatine supplementation improves muscular performance in older women. *Eur J Appl Physiol.* 2008;102:223-231.
7. Pline KA, Smith CL. The effect of creatine intake on renal function. *Ann Pharmacother.* 2005;39:1093-1096.
8. Winter BK, Fiskum G, Gallo LL. Effects of L-carnitine on serum triglyceride and cytokine levels in rat models of cachexia and septic shock. *Br J Cancer.* 1995;72:1173-1179.
9. Kraemer WJ, Volek JS, Dunn-Lewis C. L-carnitine supplementation: influence upon physiological function. *Curr Sports Med.* 2008;7:218-223.
10. Stephens FB, Constantin-Teodosiu D, Greenhuff PL. New insights concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. *J Physiol.* 2007;58:431-444.
11. Firoozrai M, Sarasgani M, Hesabi B, Bandegi A. Effect of Sports on the Reduction of Cell Membrane Susceptibility, Antioxidant Defense and Oxidative Stress. *Journal of Medical Sciences.* 2007;56:125-136. [Persian]
12. Bassit RA, Curi R, Rosa LF. Creatine supplementation reduces plasma levels of pro-inflammatory cytokines and PGE2 after a half-ironman competition. *Amino Acids.* 2008;35:425-431.
13. Akbari H, Tartibian B, Amirsasan R. Effect of L-carnitine supplementation on CRP and IL-6 during a swimming exercise in male swimmers. *Olympic Quarterly.* 2009;4:93-102.
14. Löster H, Böhm U. L-carnitine reduces malondialdehyde concentrations in isolated rat hearts in dependence on perfusion conditions. *Mol Cell Biochem.* 2001;217:83-90.
15. Rahimi R. creatine supplementation decreases oxidative DNA damage and lipid peroxidation induced by a single bout of resistance exercise. *J Strength Cond Res.* 2011;25:3448-3455.
16. Deaton CM, Marlin DJ. Exercise-Associated Oxidative Stress. *Clinical Techniques in Equine Practice.* 2003;2:278-291.
17. Basta P, Skarpan ska-Stejnborn A, Ska-Szczes'niak P. Creatine supplementation and parameters of exercise-induced oxidative stress after a standard rowing test. *Studies in Physical Cultur and Tourism.* 2006;13:23-27.
18. Waldron JEG, Kilgore TG. Concurrent creatine monohydrate supplementation and resistance training does not affect markers of hepatic function in trained weightlifters. *Journal of Exercise Physiology.* 2002;5:57-64.
19. Broad EM, Maughan RJ, Galloway SD. Carbohydrate, protein, and fat metabolism during exercise after oral carnitine supplementation in humans. *Int J Sport Nutr Exerc Metab.* 2008;18:567-584.
20. Jafaii EF, Bashiri J. The Effect of Creatine Monohydrate Loading on Downhill Running-induced Inflammatory Response in Elite Male Mountaineers. *Journal of Kerman University of Medical Sciences.* 2012;19:354-366. [Persian]
21. Haghghatdoost F, Hosseinzadeh MJ, Kabiri A, Esmailzadeh A. Effects of substituting saturated with monounsaturated fatty acid-rich diet on the serum levels of visfatin, insulin and interleukin-6 in overweight women. *Iranian Journal of Nutrition Sciences & Food Technology.* 2011;6:59-70.

22. Sachan DS, Hongu N, Johnsen M. Decreasing oxidative stress with choline and carnitine in women. *J Am Coll Nutr.* 2005;24:172-176.
23. Ferrari R, Ciampalini G, Agnoletti G, Cargnoni A, Ceconi C, Visioli O. Effect of L-carnitine derivatives on heart mitochondrial damage induced by lipid peroxidation. *Pharmacol Res Commun.* 1988;20:125-132.
24. Arockia Rani PJ, Panneerselvam C. Carnitine as a free radical scavenger in aging. *Exp Gerontol.* 2001;36:1713-1726.