Int. J. Bio-Inorg. Hybr. Nanomater., 5(3): 213-221, Autumn 2016

Serum anti-oxidation enzymes response to L-Carnitine supplementation females basketball players

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Received: 5 July 2016; Accepted: 7 September 2016

ABSTRACT: Supplementation has beneficial effects in immune system and can antioxidant enzymes activity increases during the exercise and protects the tissue against the oxidative stress. L-Carnitine is a famous Supplement and helps to protect against the oxidative stress during and after intense or prolonged exercise. The aim of this study was to investigate the effect of L-Carnitine supplementation on the blood anti-oxidation enzymes (GPX SOD, CAT and GR) response. In this semi experimental study, 20 basketball players participated. They randomly divided into two groups of supplementation and placebo respectively. Superoxide Dismutase (SOD), Catalase, Glutathione Reductase (GR) and Glutathione Peroxidase (GPX) evaluated. Supplementation groups intake 3000 mg on the day for 2 weeks. Then 2 groups participate in Basketball acute training. The data analyzed using SPSS- 20 and repeated measure ANOVA, Bonferroni and independent t-test at ($\alpha \leq 0.05$). Our findings showed a significant increase in SOD, GPX and GPX ($P<0.05$) .Based on the finding of this study, we can say that if females basketball players use L-Carnitine, this may increase their enzymes anti- oxidant .Our results suggest that the increased basal anti oxidative capacity (GPX SOD, CAT and GR) following L-Carnitine supplementation can increase the undesirable alterations of exercise-induced oxidative damage in active females.

Keywords: *L- Carnitine ; GPX ; SOD ; CAT ; Exercise ; GR ; Anti- oxidant*

INTRODUCTION

Antioxidant enzymes form the first line of defense in organisms against free radicals and toxic reactants by metabolizing them to innocuous by products (Valentine, *et al.*, 2005, Hua, *et al.*, 2015). Antioxidants are suggested as potential indirect markers of oxidative stress. Oxidative stress has been speculated to cause antioxidant consumption that results in a decline in antioxidant level (Polidori, *et al.*,2001, Hua, *et al.*, 2015). Four major enzymes, SOD, GPX, CAT and GR, were investigated here. SODs, including cytosolic copper/zinc Cu, Zn-SOD (SOD1), mitochondrial Mn-SOD (SOD2), and extracellular SOD3, are antioxidant enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide (Behndig, *et al.*,1998, Hua, *et al.*, 2015). GPX is an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage by reducing lipid

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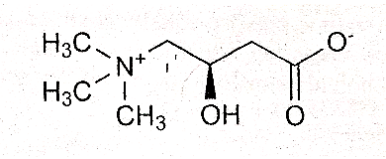

L-Carnitine

hydro peroxides to their corresponding alcohols and to reduce free hydrogen peroxide to water (Xia, *et al.*, 2015, Zhang, *et al.*, 2015, Chang, *et al.*, 2014). However, few studies have investigated the role of these antioxidant enzymes in exercise training. These data suggest that SOD, GPX, CAT and GR may play an important antioxidative role during the oxidative stress induced by exercise. Our data suggest that the oxidative cell damage may be caused by imbalance between the increased free radicals and suppressed antioxidant enzymes. Interestingly, results showed that L-carnitine reduced two oxygenases and increasing antioxidant enzymes. Findings indicate that L-carnitine provides antioxidant protection (Hua, *et al.*, 2015).

Carnitine (3-hydroxy-4-N-trimethylammonium butyrate) is a compound that can be either endogenous, synthesized by the liver and kidneys, both exogenous, introduced with the diet, in particular through meat and dairy products. Carnitine supplementation has been hypothesized to improve exercise performance in healthy humans through various mechanisms and several physiological functions; enhanced muscle fatty acid oxidation, modification of training responses, and altered muscle fatigue resistance.

L-Carnitine (a non-protein amino acid β-hydroxy-γ-trimethyl-amino-butyric acid), suppresses inflammatory responses in human and homeostasis is affected by exercise in a well-defined manner because of the interaction of the carnitine – acyl carnitine pool with key metabolic pathways. Carnitine has a dual role as it is required for long-chain fatty acid oxidation, and also shuttles accumulated acyl groups out of the mitochondria, but also Carnitine effect on immune system and antioxidant enzymes. It is believed that the amount of antioxidant enzymes activity increases during the exercise and protects the tissue against the oxidative stress. L- Carnitine supplementation has beneficial effects in immune system. L- Carnitine has played an important role as modulator of cellular stress response (Kendler, 1986). L-Carnitine may use as a supplement to treat cardiovascular or liver disease (Ho, *et al.*, 2013, Mingorance, *et al.*, 2011, Schnabel, 2007, Johri, 2014). L-Carnitine or 3-hydroxy-4-N-trimethyl ammonium butyrate is an endogenous compound which has sev¬eral physiological functions. L-Carnitine is involved in the transfer of long-chain fatty acids across the inner matrix membrane of mitochondria. It also regulates acetyl storage and transfer in mitochondria, cells, and between organs (Bremer, 1983, Rani, 2002). Many in vitro and animal studies have reported that LC is a free radical scavenger, which protects antioxidant enzymes from oxidative damage (Bremer, 1983, Kolodziejczyk, *et al.*, 2011). In a human study, Cao (Cao, 2011) administered LC supplement (2000 mg/d) to healthy volunteers and observed that LC significantly increased the levels of antioxidant enzymes activities, suggesting that LC might be useful for treating chronic illnesses (Hua, *et al.*, 2015). Research looking at L-Carnitine supplementation and endurance exercise has so far been conflicting with some studies finding improved exercise performance whilst others have failed to find improved exercise performance. However, most of these studies have looked at short term acetyl-l-carnitine supplementation (Ribas, *et al.*, 2014). In one study, researchers looked at the long term supplementation of L-Carnitine (2g/daily of L-Carnitine over a 24 week period), increased work output during a 30 minute all-out exercise test, preserved muscle glycogen during low intensity exercise and reduced the reliance on anaerobic ATP production during high intensity exercise (Bremer 1983). The exact mechanisms by which L-Carnitine may improve recovery following strenuous exercise is not completely clear but is likely to be a combination of a number of factors, including . L-Carnitine's is known to possess strong antioxidant properties and therefore helps to protect against the oxidative stress during and after intense or prolonged exercise .Supplementation with L-Carnitine can increase blood flow, and therefore enhance oxygen delivery, to the working muscles. This may also enhance post exercise recovery (Kendler, 1986). Superoxide Dismutase (SOD), catalase (CAT) Glutathione reductase (GR), Glutathione peroxidase (GPx) are the enzymes that release in blood (Marklund, 1974, De Sotomayor, 2007). Intakes of dietary antioxidants are great importance for the

protective effects of enzymatic antioxidant defenses (Gaby, 2010). L-Carnitine supplementation at a dose of 1000 mg/d was associated with a significant reduction in oxidative stress and an increase in antioxidant enzymes activities in CAD patients. L- Carnitine supplements will increase anti-oxidation capacity (Lee, *et al.*, 2014). Single dose administration of L-Carnitine increases activities of antioxidant enzymes and the total antioxidant capacity in healthy subjects. It may be useful as a supplementary therapy for chronic illnesses involving excessive oxidative stress (Cao, 2011). Researchers found a gradual increase in plasma concentrations of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase and total anti oxidative capacity (T-AOC) in the first 3.5 h following L-Carnitine administration. The plasma concentrations of SOD, GSH-Px, and Catalase returned to baseline levels within 24 h. A positive correlation was found between L- Carnitine concentration and the antioxidant index of SOD, GSH-Px and Catalase (Cao, 2011). This study aims to determine the effect of L-Carnitine supplementation on some of antioxidant (GPx, SOD, CAT,GR) indices responses in serum females players , after acute one-session Basketball training.

MATERIAL AND METHODS

Twenty females Basketball Players into an experimental, randomized design were divided in two homogeneous supplement (mean age 25±3 years, body mass 52±4 and VO2max 38±4ml/kg-1/min) and placebo groups (mean age 24 ± 2 years , body mass 54 ± 3 kg, and VO2max 39/5 ± 2 ml/kg-1/min) (3g on day L- carnitine or Dextrose). Subjects were informed as to the potential risks associated with participation in the study before obtaining their written informed consent to participate. The study was carried out in accordance with the IAU and approved by the Ethical Committee. Liquid L-Carnitine (3.0 g) was administered orally as 3 single doses in subjects. After 14 days of supplementation, all subjects were participated in the Basketball acute training protocol. The laboratory area during all trials was 23 ± 2°C. Paired samples of antecubital venous blood were taken at three phases (baseline, after supplementation period and immediately after the exercise). Plasma concentration enzymes were detected by HPLC. In this trial, we have demonstrated that LC administered at a dose of 3000 mg/d for 2 weeks significantly increased the antioxidant enzymes activities. After 2 weeks of LC supplementation at a dose of 3000 mg/d can increase the activities of GPX by 36%, SOD by 47%, and CAT by 12% and GR 7%. Increased RBCs antioxidant enzymes activities can provide a protection against oxidative damage to the endothelial cells. LC was found to be an effective antioxidant agent in basketball players and prevent through its antioxidant property.

STATISTICAL ANALYSES

The data were analyzed using SPSS software (version 22). The normal distribution of variables was tested by the Kolmogorov-Smirnov test. Differences in subjects' demographic data and hematological measurement data between the placebo and LC groups were analyzed by one way analysis of variance (ANOVA), repeated measures test and Bonferroni test was used for post hoc multiple comparisons among means .The paired t-test was used to analyze the data within each group before (baseline) and after intervention (week 2). Results were considered statistically significant at $P < 0.05$. Values presented in the text are means ± standard deviations (SD). All samples were tested in duplicate and all data are expressed as Means ± SD.

STUDY PARTICIPANT CHARACTERISTICS

Table 1 shows the demographic data and health characteristics of the subjects. There were no significant differences between the two groups with respect to age and anthropometric measurements.

RESULTS AND DISCUSSION

The results showed that the 14-days L-Carnitine intake had significant effect on the basal anti oxidative enzymes (GPX, SOD, CAT, GR) capacity ($P \leq 0.05$).

Table 1. Demographic data and health characteristics of the subjects.

Factors / Groups	Dextrose	L- Carnitine	P- value
Age (year)	22.28±1.71	23.28±1.12	0.34
Weight (kg)	58.89±3.21	59.13±4.12	0.23
Height (cm)	165.12±2.51	167.19±3.15	0.16
Body Mass (kg/m^2)	22.12±3.93	23.67±3.29	0.48
Body Fat (%)	17.12±2.39	16.08±1.14	0.19
Vo^{2}max (ml/kg^1)	40.88±2.18	38.65±3.96	0.52

Moreover, Basketball acute exercise significantly effected anti oxidative power (P≤0.05). Therefore, supplementation L-Carnitine and performing special basketball exercises result in improvement and fortification of antioxidant system against the oxidative stress produced during exercise. Blood samples were collected baseline , after supplementation and immediately following training and used for determination of antioxidant enzymes including GPX ,CAT,GR and SOD enzymes activity. The antecubital serum enzymes activity concentration was elevated .Mean ±SD and ANOVA results of antioxidant enzymes have shown in Table 1 and (Figs. 1 and 2). ANOVA results showed that GPX and SOD activity in supplementation group was increased significantly. CAT activity increased and were significant differences , but Bonferroni test didn't significant, also, there was no significant differences in GR level between all 2 groups. These findings suggested that L-Carnitine consumption 2 weeks before training may be affects antioxidant enzymes concentration and promotes antioxidant capacity of players during heavy training. As a result, it seems clear that LC has a protective effect, which could be ascribed to its antioxidant capacity (Gülçin, 2006) have reported that LC might be a good antioxidant. LC has an effect on free radicals (such as 1, 1-diphenyl-2-picryl-hydrazyl radical, superoxide anion radical, hydrogen peroxide) scavenging. LC might interfere with the reactive oxygen species formation and chelate the metal ferrous ions (Gülçin, 2006). In the LC molecule, the carbonyl group can stabilize the free radicals formed on α-carbon with conjugation, and it

Table 2. Mean ±SD and ANOVA results of antioxidant enzymes.

Factors	Fase / Supplements	Base	After supplementation	Immediately After exercise
GPX (U/gr Hb)	Dextrose	24.45±1.68	26.73±1.19	23.12±1.68
	L-Carnitine	23.35±0.56	43.89±1.13	39.91±1.46
	P value	0.08	0.000*	0.07
SOD (U/gr Hb)	Dextrose	1161.45±34.19	1167.49±345.19	1152.73±412.67
	L-Carnitine	1092.52±267.29	1189.76±249.18	1181.59±346.65
	P value	0.06	0.006*	0.11
CAT (k/gr Hb)	Dextrose	276.82±50	279.46±39	271.76±34
	L-Carnitine	281.45±46	287.78±56	282.11±53
	P value	0.11	0.007	0.013
GR (U/gr Hb)	Dextrose	13.92±1.56	15.45±2.11	14.29±2.17
	L-Carnitine	15.35±1.98	18.59±2.34	17.70±1.99
	P value	0.13	0.09	0.14

*: (P ≤ 0/ 05)

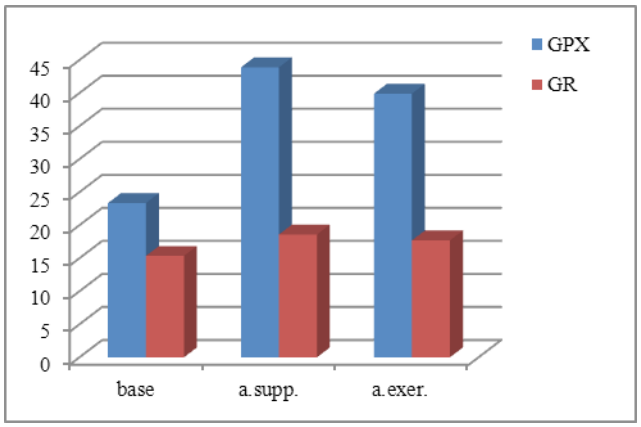

Fig. 1. Mean of GPX and GR in baseline, after supplementation and after exercise

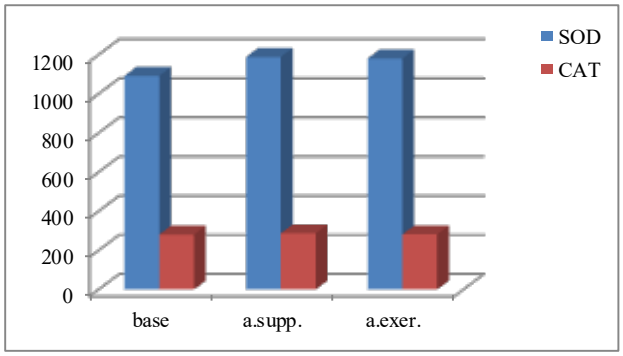

Fig. 2. Mean of SOD and CAT in baseline, after supplementation and after exercise

protects plasma components against the toxic action of reactive oxygen species and reactive nitrogen species (Gülçin, 2006 , Kolodziejczyk, *et al.*, 2011). In addition, LC is also an essential cofactor of carnitine palmitoyl transferase 1 (CPT1), which allows fatty acid transport into mitochondria and the incorporation of long chain fatty acids into the β-oxidation cycle to obtain acetyl-CoA (Lysiak, *et al.*,1988, Mingorance , *et al.*, 2011), and these substances enter the tricarboxylic acid (TCA) cycle to synthesize adenosine triphosphate (ATP). At this step of ATP synthesis, a large amount of oxygen is consumed, and the oxygen is reduced to water at the end of the TCA cycle. Then, oxygen concentration decreases and reactive oxygen species formation is also reduced (Gülçin, 2006, Botham, *et al.*, 2012). We suggest that LC could be acting as a buffer for excessive acetyl groups in mitochondria, decreasing mitochondrial superoxide production during hypoxia or substrate excess, especially in the ischemic tissues .Oxidative stress might play a crucial role. Antioxidant therapy might prove beneficial in combating clinical problems (Dhalla, *et al.*, 2000) and protect against excessive oxidative stress (Lee, *et al.*, 2014). Different physical activities effect on anti-oxidative system (Richards, *et al.*, 1988). Varity, period and intensity of activities are necessary in health improvement. This study was carried out to determine the effect of supplementation L-Carnitine on serum level of SOD (superoxide dismutase), Catalase, GR and GPX in female basketball players (Gülçin, 2006, Marklund, *et al.*, 1974). There was an increase in plasma concentrations of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and Catalase, following L-Carnitine administration. The plasma concentrations of SOD, GPx, Catalase GR returned to baseline levels within 2-4 h. A positive correlation was found between L-Carnitine concentration and the antioxidant index of SOD, GPX, Catalase and GR. In conclusion, L-Carnitine increases activities of antioxidant enzymes in healthy subjects (Cao, *et al.*, 2011). Moreover, it was observed there is significant difference between anti oxidative enzymes of two groups ($P > 0.05$). Therefore, L-Carnitine supplementation and performing basketball exercises result in improvement and fortification of antioxidant system against the oxidative stress produced during exercise. These results show that basketball exercise training can cause oxidative and stress in blood and another tissue, supplementation treatment can either ameliorate or enhance effect of anti-oxidation, by activating defense systems (Sener, *et al.*, 2004, Richards, *et al.*, 1998). Free radicals are produced during cellular metabolism and have key roles as regulatory mediators in signaling processes (Gülçin, 2006). Oxidative stress reflects an imbalance between production of reactive oxygen species and an adequate antioxidant defense. This adverse condition may lead to cellular and tissue damage of components, and is involved in different physio-pathological states, including aging, exercise, inflammatory, cardiovascular and neurodegenerative diseases (Lee, *et al.*, 2014, Flanagan, *et al.*, 2010, Johri, *et al.*, 2014). In particular, the relationship between exercise and oxidative stress is extremely complex, depending on the mode, intensity and duration of exercise. Regular moderate training appears beneficial for oxidative stress and health. Supporting endogenous defenses with additional oral antioxidant supplementation may represent a suitable noninva-

sive tool for preventing or reducing oxidative stress during training (Kolodziejczyk, *et al.*, 2018 Gülçin, 2006). Antioxidant supplementation may be warranted in particular conditions, when athletes are exposed to high oxidative stress or fail to meet dietary antioxidant requirements (Kendler, 1986). Aim of this study is to discuss the evidence on the relationship between exercise and oxidative stress, and the potential effects of L-Carnitine in women basketball players. Many studies have sought to determine whether supplements would benefit those who exercise regularly.

But many studies found no advantage of supplements on exercise performance, but there is little theoretical basis to believe that they would have an effect (Harrison, et al., 2013, Gaby, 2010, Kendler, 1986). Moreover, several factors govern human performance, thus making it difficult to detect effects of a supplement intervention. Other studies examined whether supplements reduced measures of oxidative stress ,and just as the studies to examine exercise-induced oxidative stress produced varied results, so did these studies regarding supplementation. The type of supplement, timing of the supplement, and the outcome measures were different among the studies, making any overall interpretation difficult (Kolodziejczyk, *et al.*, 2011, De Sotomayor, *et al.*, 2007, Kendler, 1986). Antioxidant enzymes, such as SOD, Catalase, and GPX, constitute a natural defense system against the activity of oxidants. The survival of an organism may depend on the ability of the organism to overcome the toxic effects of oxidants (ROS). SOD catalyzes the dismutation of the superoxide radical into hydrogen peroxide (H_2O_2).Studies showed that Carnitine supplementation enhances the dismutation of superoxide radicals by increasing the levels of SOD activities (Marklund, *et al.*, 1974, De Sotomayor, *et al.*, 2007). Apparently L-Carnitine promotes energy production. The energy-promoting action of L-Carnitine was confirmed .An increase in the activity of Catalase after supplementation was observed, confirming that Carnitine provides reducing equivalents necessary for converting Catalase from the inactive form to the active form (Kendler, 1986). Carnitine supplementation increased overall antioxidant enzyme status as a function of the duration of treatment, thus decreasing the levels of free radicals available. Carnitine can also act as a chelating agent by decreasing the concentration of cytosolic iron, which plays a very important role in free radical chemistry. Carnitine and its esters protect cells from ROS damage both by inhibiting free radical propagation and by contributing to the repair of oxidized membrane phospholipid (Rani , *et al.*, 2007). In the present study, there was a significant positive correlation between the levels of LC and antioxidant enzymes activities after supplementation. LC was found to be an effective antioxidant agent in cardiovascular disease models and prevent endothelial dysfunction through its antioxidant property (As a result, it seems clear that LC has a protective effect against oxidants which could be ascribed to its antioxidant capacity. Gülçin have reported that LC might be a good antioxidant. LC has an effect on free radicals (such as 1, 1-diphenyl-2-picryl-hydrazyl radical, superoxide anion radical, hydrogen peroxide) scavenging. LC might interfere with the reactive oxygen species formation and chelate the metal ferrous ions (Gülçin, 2006). Oxygen concentration decreases and reactive oxygen species formation is also reduced (Bremer, 1983). LC could be acting as a buffer for excessive acetyl groups, decreasing superoxide production during hypoxia or substrate excess, especially in the ischemic tissues (Kolodziejczyk, *et al.*, 2011). Reduced concentrations of L- Carnitine may occur due to the combination of this compound to the accumulating toxic metabolites, especially organic acids, or as a result of protein restricted diets. Thus, L- Carnitine supplementation may be useful not only to prevent tissue deficiency of this element, but also to avoid oxidative damage secondary to increased production of reactive species. However further studies are required to better explore this potential (Ribas, *et al.*, 2014). Additional trials integrating physiologic, biochemical, and pharmacologic assessments are needed to definitively clarify any effects of L-Carnitine on exercise and anti-oxidative enzymes in healthy persons. L-Carnitine acts as a carrier for fatty acids across the inner mitochondrial membrane necessary for subsequent beta-oxidation and ATP production. Besides its important role in the metabolism of lipids, l-carnitine is also a potent antioxidant (free radical scavenger) and thus may protect tissues from oxidative damage. L-carnitine may be involved in the reduction of oxidative damage by

antioxidant enzymes. L-carnitine supplementation is useful by increasing anti-oxidant enzymes, to avoid oxidative damage secondary to increased production of reactive species in these diseases, may also be beneficial in preventing damage derived from oxidative injury. Studies showed the effect of LC supplementation on metabolic indexes, laboratory parameters, and significant clinical outcomes. But the most important role of L-Carnitine is:

L-Carnitine supplements can help boost carnitine levels, L-Carnitine is an antioxidant In the brain, L-Carnitine can be taken to help treat peripheral nerve injury, L-Carnitine supplements is that they may help to reduce the side effects of various drug treatments, although some carnitine based supplements are marketed for weight loss, there is no clear evidence to support this and l-carnitine supplements are often bought to enhance general wellness, due to their properties as a powerful antioxidant which reduces oxidative stress in the body.

CONCLUSIONS

In conclusion, we have demonstrated that LC supplementation at a dose of 3000 mg/d significantly reduced oxidative stress and increased antioxidant enzymes activities in basketball players and sing LC supplements to increase their anti-oxidation capacity.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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