Asian Journal of Chemistry Vol. 21, No. 3 (2009), 2027-2031

Effects of Acute L-Carnitine Supplementation on Lipid Peroxidation and Nitric Oxide Level

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The purpose of this study was to determine the effects of acute L-carnitine supplementation on blood malondialdehyde (MDA) level as a marker of lipid peroxidation and nitric oxide (NO) level. With this aim, 8 male and 8 female total 16 national badminton players participated into this study voluntarily. Astrand protocol was used to determine the maximal oxygen consumption. Each subject received an acute dose (2 g L-carnitine before 1 h of exercise) and placebo in a randomized, double-blind crossover design. Dietary intake and exercise were replicated for 4 d prior to each trial. Blood samples were drawn by venipuncture before and immediately after exercise. No significant differences were found between measurements of with and without L-carnitine supplementation of participants as regards to all measured parameters. The result of this study shows that acute L-carnitine supplementation before 1 h of the exhaustive exercise does not affect lipid peroxidation and nitric oxide levels.

Key Words: Exercise, L-Carnitine, Nitric oxide, Malondialdehyde.

INTRODUCTION

Physical exercise leads to increased oxygen consumption, which in turn generates reactive oxygen/nitrogen species (RONS)[1]. Production of RONS in quantities that overwhelm the endogenous antioxidant defense system has been referred to as oxidative stress[2]. Lipid peroxidation is the most common index of oxidative stress and malondialdehyde, the last product of lipid peroxidation, is routinely used as a marker of this process.

Nitric oxide is a free radical produced in biological systems. Disturbances in the metabolism of nitric oxide (NO) can be related to many pathophysiological events, in particular, the oxidative stress of sports activity[3]. Jay-Gerin and Ferradini have postulated that NO-reductase or NO-dismutase regulates the high local NO concentrations released within NO-generating cells in response to oxidative stress[4].

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Flow-mediated release of NO is also believed to be important for exercise-induced vasodilatation, as suggested by the work of Gilligan and co-workers[5]. This result suggests that shear stress during the exercise may increase production of NO in normal subjects.

L-Carnitine has been described as a conditionally essential nutrient for human beings. It is required for the transport of long-chain fatty acids into the mitochondria for fuel and also for the maintenance of key proteins and lipids of the mitochondria at sufficient levels for maximum energy production[6]. Recently growing attention has been focused on the antioxidant effects of L-Carnitine[7,8] and an antioxidant effect has been described[9]. L-Carnitine prevents oxidative stress and regulates nitric oxide, the cellular respiration[10] and the activity of enzymes involved in defense against oxidative damage[10].

The aim of this study was to investigate the effects of L-carnitine on lipid peroxidation and NO levels.

EXPERIMENTAL

Test subjects: This study was carried out on 8 elite female badminton players with an average age, height and body weight of 20.38 ± 2.50 years, 164.00 ± 5.04 cm and 58.13 ± 5.49 kg and 8 elite male badminton players with an average age, height and body weight of 25.38 ± 3.20 years, 180.25 ± 5.42 cm and 72.38 ± 8.86 kg (Table-1). All players were playing in Turkish National Teams. All experimental procedures were approved by the Gazi University ethical committee. All subjects were asked to give both verbal and written consent prior to participation.

TABLE-1
PHYSICAL CHARACTERISTICS OF THE PARTICIPANTS

Variables	Age (years)	Body weight (kg)	Height (cm)	Experience (years)
Male (n = 8)	25.38 ± 3.20	72.38 ± 8.86	180.25 ± 5.42	7.38 ± 3.34
Female (n = 8)	20.38 ± 2.50	58.13 ± 5.49	164.00 ± 5.04	5.63 ± 1.92

The participants were subjected to the test protocol twice before and after 2 g of oral L-carnitine intake (Sigma Chemical Co.). They were asked to refrain from making excessive activity and alcohol and caffeine consumption 24 h before the measurements. The participants were given 4 d of resting period between 2 measurements.

Exercise protocol: Astrand protocol was used to determine the maximum VO_2 values. The speed of the treadmill was kept constant at 9.7 km/h and the incline was adjusted at 0 degrees initially and was increased by 2 % every 2 min and the participants were asked to run on it till the exhaustion[11].

Taking blood samples and analysis: Venous blood samples were drawn by antecubital venipuncture before the bout and immediately after the bout (within 1 min). The blood was immediately centrifuged at 1500 RCF for 10 min at 4 ºC and the plasma was separated and stored in Eppendorf tubes at -70 ºC for subsequent use. Plasma samples were used for measurements of malondialdehyde and NO level.

NO Measurement: The NO levels were measured in plasma as nitrites using the modified Griess reaction after converting nitrates to nitrites with vanadium chloride. Standard curves for sodium nitrite were prepared. Values were calculated with standard calibration plots for $NaNO_2$ and $NaNO_3$ as previously described[12,13].

Lipid peroxidation: Lipid peroxidation was quantified by measuring the formation of thiobarbituric acid reactive substances as described previously by Kurtel *et al.*[14]. Aliquots (0.5 mL) were centrifuged and the supernatants were added to 1 mL of a solution containing 15 % (w/v) tricarboxylic acid, 0.375 % (w/v) thiobarbituric acid and 0.25 N HCl. Protein precipitate was removed by centrifugation and the supernatants were transferred to glass test tubes containing 0.02 % (w/v) butyrate hydroxytoluene to prevent further peroxidation of lipids during subsequent steps. The samples were then heated for 15 min at 100 ºC in a boiling water bath, cooled and centrifuged to remove the precipitant. The absorbance of each sample was determined at 532 nm. Lipid peroxide levels were expressed in terms of malondialdehyde equivalents using an extinction coefficient of 1.56×10^5 mol^{-1}.

Statistical analysis: Values are expressed as the mean ± SD. The data were evaluated by the use of SPSS 13.0 for Windows. Statistical analyses were performed with Wilcoxon signed ranks test. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Tables 2 and 3 demonstrated that the lipid peroxidation and NO level were increased both in female and male volunteers subjects after exercise in plasma. But this increase was not statistically significant.

TABLE-2
MEASUREMENT OF MDA (nmol/mL) AND NO (µmol/L) VALUES BEFORE AND AFTER EXERCISE WITH L-CARNITINE AND PLACEBO OF THE FEMALE PARTICIPANTS (n = 8)

		Before exercise	After exercise
MDA	L-carnitine	4.72 ± 2.7	7.12 ± 3.7
	Placebo	4.13 ± 2.4	8.42 ± 4.5
NO	L-carnitine	112.98 ± 36.5	145.25 ± 65.9
	Placebo	86.16 ± 11.7	106.73 ± 32.9

TABLE-3
MEASUREMENT OF MDA (nmol/mL) AND NO (µmol/L) VALUES BEFORE AND AFTER EXERCISE WITH L-CARNITINE AND PLACEBO OF THE MALE PARTICIPANTS (n = 8)

		Before exercise	After exercise
MDA	L-carnitine	6.80 ± 3.3	9.47 ± 4.1
	Placebo	4.77 ± 3.2	5.63 ± 3.2
NO	L-carnitine	114.57 ± 42.9	126.60 ± 51.5
	Placebo	86.48 ± 12.8	107.09 ± 24.2

The data from the present study do not support an effect of a single dose (2 g) with L-carnitine on lipid peroxidation and NO metabolism. No significant effects of acute supplementation of L-carnitine were observed in either males or females. Vascular formation of NO is directly facilitated by increased shear stress[15]. During a session of physical exercise, cardiac output increases and blood redistribute to the exercising muscles. The exercise-induced increase of blood flow elicits an increase in shear stress[16], thereby providing a possible coupling between exercise and endogenous NO formation. Although the role of endothelium-derived NO in acute exercise has not been fully resolved, exercise training involving repetitive bouts of exercise over weeks or months up-regulates endothelial NO bioactivity[17]. It has also been reported that NO is increased in venous plasma after prolonged running, cycling[18] and after incremental cycling exercise to VO_2 max[19]. In contrast, some other studies have also shown no change of the NO metabolism following an incremental treadmill test to exhaustion in healthy human subjects[20-22]. In the present study NO level were increased both in female and male volunteers subjects after exercise in plasma. But this increase was not statistically significant.

L-Carnitine, as an antioxidant, can protect antioxidant enzymes from further peroxidative damage[10]. Hence, supplementation of antioxidant L-carnitine may result in attenuation of lipid peroxidation. Dayanandan *et al.*[23] have reported that the protective effect of carnitine supplementation against lipid peroxidation in tissues of atherosclerotic rats but present study depicts that L-carnitine treatment (300 mg/kg body weight/day) for 7 and 14 d caused significant reduction in the tissue lipid peroxidation. Also studies have shown that carnitine supplementation enhanced fatty acid oxidation in skeletal muscle[24,25].

L-carnitine is essential for the β-oxidation of fatty acids in mitochondria to generate ATP[26]. Chang *et al.*[26] also reported that L-carnitine effectively prevents mitochondrial injury deriving from oxidative damage. Cellular processes affected by mitochondrial degeneration include detoxification, calcium homeostasis. Mitochondrial degeneration may eventually cause inadequate energy production, thereby compromising vital dependent reactions. It is known that L-carnitine shows its protective effects on mitochondria and membrane damage, thus amount of ATP production increase by L-carnitine. Cells' vital functions work effectively under enough energy productions, like Ca-ATP-ase enzymes, endoplasmic reticulum and mitochondria.

There are some studies on humans determining that the pre-exercise supplementation of different doses L-carnitine (1-6 g) had no effect on maximal exercises performance[27-29]. In many studies especially high dose prolonged administration of L-carnitine resulted in a moderate improvement in the exercise performance and maximum VO_2 ($MaxVO_2$) values of unprofessional and non-elite sports people. However, there are also studies reporting that carnitine supplementation caused no change in $MaxVO_2$ and lactate accumulation in high intensity exercise[30].

Performance parameters were generally evaluated in human studies with acute and chronic L-carnitine supplementation. Lipid peroxidation was found to be decreased in chronic L-carnitine supplementation studies where oxidative stress was evaluated[6]. In this study, acute dose of L-carnitine supplementation was made; however, there was no difference between the two observations. Results of this study show that acute L-carnitine supplementation before 1 h of the exhaustive exercise does not affect lipid peroxidation and nitric oxide levels.

REFERENCES

1. M.I. Urso and P.M. Clarkson, *Toxicology*, **189**, 41 (2003).
2. R.J. Bloomer, A.H. Goldfarb and M.J. Mckenzie, *Med. Sci. Sports Exerc.*, **38**, 1098 (2006).
3. D.L. Lawson, L. Chen and J.L. Mehta, *Am. J. Cardiol.*, **80**, 1640 (1997).
4. J.P. Jay-Gerin and C. Ferradini, *Biochimie*, **82**, 161 (2000).
5. D.M. Gilligan, J.A. Panza, C.M. Kilcoyne, M.A. Waclawiw, P.R. Casino and A.A. Quyyumi, *Circulation*, **90**, 2853 (1994).
6. S. Savitha, J. Tamilselvan, M. Anusuyadevi and C. Panneerselvam, *Clin .Chim. Acta*. **355**, 173 (2005).
7. A. Arduini, *Am. Heart J.*, **123**, 1726 (1992).
8. L.A. Calo, E. Paagnin, D.A. Davis, A. Semplicini, R. Nicolai, M. Calvani and A.C. Pessina, *Int. J. Cardiol.*, **107**, 54 (2006).
9. G. Sener, K. Paskaloglu, H. Saturoglu, I. Alican, A. Kacmaz and A. Sakarcan, *J. Cardiovasc Pharmacol.*, **43**, 698 (2004).
10. I. Gulcin, *Life Sci.*, **78**, 803 (2006).
11. J. Kang, E.C. Chaloupka, M.A. Mastrangelo, G.B. Biren and R.J. Robertson, *Eur. J. App. Physiol.*, **84**, 291 (2001).
12. L.C. Green, D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnok and S.R. Tannenbaum, *Anal. Biochem.*, **126**, 131 (1982).
13. K.M. Miranda, M.G. Espey and D.A. Wink, *Nitric Oxide*, **5**, 67 (2001).
14. H. Kurtel, D.N. Granger, P. Tso and M.B. Grisham, *Am. J. Physiol.*, **263**, 573 (1992).
15. J.P. Cooke, E. Rossitch, N.A. Jr Andon, J. Loscalzo and V.J. Dzau, *J. Clin. Invest.*, **88**, 1663 (1991).
16. R.L. Van Citters and D.L. Franklin, *Circulation Res.*, **24**, 33 (1969).
17. A. Maiorana, G. O'Driscoll, R. Taylor and D. Green, *Sports Med.*, **33**, 1013 (2003).
18. L. Jungersten, A. Ambring, B. Wall and A. Wennmalm, *J. Appl. Physiol.*, **82**, 760 (1997).
19. K. Node, M. Kitakaze, H. Sato, Y. Koretsune, Y. Katsube, M. Karita, H. Kosaka and M. Hori, *Am. J. Cardiol.*, **79**, 526 (1997).
20. Y. Komiyama, Y. Kimura, N. Nishimura, K. Hara, T. Mori, K. Okuda, M. Munakata, M. Masuda, T. Murakami and H. Takahashi, *Clin. Exp. Hypertens.*, **19**, 363 (1997).
21. C.M. St Croix, T.J. Wetter, D.F. Pegelow, K.C. Meyer and J.A. Dempsey, *Am. J. Respir. Crit. Care Med.*, **159**, 1125 (1999).
22. J.J. Poveda, A. Riestra, E. Salas, M.L. Cagigas, C. Lopez-Somoza, J.A. Amado and J.R. Berrazueta, *Eur. J. Clin. Invest.*, **27**, 967 (1997).
23. A. Dayanandan, P. Kumar and C. Panneerselvam, *J. Nutr. Biochem.*, **12**, 254 (2001).
24. R.F. Bacurau, F. Navarro, R.A. Bassit, M.O. Meneguello, R.V. Santos, A.L. Almeida and L.F. Costa Rosa, *Nutrition*, **19**, 337 (2003).
25. J. Karanth and K. Jeevaratnam, *Int. J. Vitam. Nutr. Res.*, **75**, 333 (2005).
26. B.J. Chang, M. Nishikawa, S. Nishiguchi and M. Inoue, *Int. J. Cancer*, **113**, 719 (2005).
27. E.P. Brass, C.L. Hoppel and W.R. Hiat, *Clin. Pharmacol. Ther.*, **55**, 681 (1994).
28. R. Nuesch, M. Rosetto and B. Martina, *Drugs Exp. Clin. Res.*, **25**, 167 (1999).
29. H. Eroglu, O. Senel and N.A. Guzel, *Neuroendocrinol. Lett.*, **29**, 1 (2008).
30. H. Karlic and A. Lohninger, *Nutrition*, **20**, 709 (2004).

(*Received*: 14 March 2008; *Accepted*: 12 November 2008) AJC-7023