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Full Length Research Paper

Investigation of carbonic anhydrase levels under exercise and hyperthermic stress in rats given L-carnitine

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L- carnitine is a co-factor of the enzymatic system involved in long chain fatty acid transport across the mitochondrial membrane. L-carnitine also modulates the metabolism of coenzyme-A (CoA). The functions of L-carnitine in skeletal muscle are critical to sustaining normal bioenergetics during exercise. Therefore, it is not surprising that the use of supplementary carnitine to improve physical performance has become widespread in recent years. Carbonic anhydrases (CAs, EC 4.2.1.1) are widespread enzymes in all organisms, catalyzing CO_2 hydration to bicarbonate and protons. It is known that CA inhibition alters skeletal muscle contractile properties, utilization of metabolic substrates, and accumulation of metabolic intermediates and end products, especially during exercise. In this study, changes in carbonic anhydrase (CA) levels due to exercise and hyperthermic stress in rats were investigated. For this purposes, 24 healthy Spraque Dawley male rats were divided into four groups: Exercise group 1 (at 38°C), Exercise group 2 (control group at 28°C), L-carnitine + Exercise group 3 (at 38°C), L-carnitine + Exercise group 4 (L-carnitine + control group at 28°C). The results of this study indicated that CA inhibition significantly decreased at L-carnitine + Exercise group 4 (at 28°C) (P<0,01) and Exercise group 1 (at 38°C) (P<0.005). According to L-carnitine + Exercise group 3 (at 38°C). It may be considered that L-carnitine does not have a protective role in exercise done under hyperthermic conditions.

Key words: L-carnitine, hyperthermic stress, carbonic anhydrase, enzyme, exercise.

INTRODUCTION

L-carnitine is an amino acid derivative whose primary roles in the human body are in transporting long-chain fatty acids into the mitochondria for use as a fuel and buffering excess acyl-CoA accumulation within mitochondria, and the site of β-oxidation (Broad, 2006; Gülçin, 2006a; Tunstall, 2002). It is known that L-carnitine has powerful antioxidant activity (Gülçin, 2006a). Antioxidant activity of pure molecules was extensively studied recently (Ak and Gulcin, 2008; Gülçin, 2006b, 2007, 2008a, 2008b, 2010; Gülçin and Daştan, 2007; Koksal et al., 2009) and gained great importance (Balaydın et al., 2010; Gülçin et al., 2002, 2003, 2004a, 2004b, 2005a, 2005b, 2006a, 2006b, 2008a; 2010a; Talaz et al., 2009). It is still a matter of debate whether the administration of L-carnitine improves performance of intensive endurance exercise (Brass, 2000). As reported in the majority of studies, L-carnitine has been shown to induce a significant postexercise decrease in plasma lactate, which is formed and used continuously under fully aerobic conditions. Recent data have indicated that L-carnitine plays a decisive role in the prevention of cellular damage and favorably affects recovery from exercise stress (Karlic and Lohninger, 1996; Stephens et al., 2007). Heinonen and Takala (1994) emphasized that carnitine depletion of 48% has no effect on palmitate oxidation, exercise capacity, or nitrogen balance in the rats studied. Greig et al. (1987) claimed that in researches they carried out with various different exercises, taking L-carnitine before exercise or increasing of acute carnitine has no effect on performance.

The metalloenzyme Carbonic anhydrases (CAs, EC 4.2.1.1) is an enzyme that catalyzes the interconversion of carbon dioxide to bicarbonate and protons (Hisar et al., 2005a; 2005b; 2006; Cankaya et al., 2007; Supuran, 2008; Innocenti et al., 2010a; 2010b). This enzyme, presented in most tissues including erythrocytes, involves in a wide range of physiological and biochemical processes. Thereby, it plays an important role in CO_2 transport, acid-base balance and fluid secretion and absorption, and ventilatory control (ArasHisar et al., 2004; Henry, 1996; Scheuermann et al., 2000; Şentürk et al., 2010; Coban et al., 2009). Carbonic anhydrase can be situated at several organs and tissues in the body, such as the kidney, erythrocytes, the nervous system, and pulmonary and muscle tissue, (Swenson and Hughes, 1993; Swenson et al., 1993; Swenson, 1998; Ozturk Sarikaya et al., 2010; Wagenaar et al., 1998). Skeletal muscle contains 2 isoforms of Carbonic anhydrase, III and IV (Carter et al., 1979). Isoform III protects against free radical damage and controls the intermediary metabolism of glucose and fat, whereas Isoform IV facilitates carbon dioxide removal (Geers, 1991). Carbonic anhydrase catalyzes the reversible hydration and dehydration of carbon dioxide, a product of cellular aerobic energy production (Vallee, 1993). Thus, the ubiquitous distribution of carbonic anhydrases in mammalian tissues and its heterogeneous roles in cellular energy metabolism is immanent.

Physical exercise is an activity presenting systematic repetitions of oriented movements featured with consequent increase in the oxygen intake due to muscular demand, thus generating work. The physical exercise causes a series of physiological responses in the body systems. Regular exercise training improve cardiovascular function and pulmonary capacity - presenting as typical examples the rest relative bradycardia, the muscular hypertrophy, the physiological left ventricular hypertrophy and the increase on the maximal oxygen intake (VO_2 maximum), increased the blood flow into the skeletal muscles and into the cardiac muscle, and increase sarcolemmal transport and mitochondrial β-oxidation of fatty acids and plasma-borne delivery (Adegoke and Arogundade, 2002; Monteiro et al., 2004; Radak et al., 2001). It also induces mitochondrial biogenesis in skeletal muscle (Higashida, 2008). It is known that CA inhibition alters skeletal muscle contractile properties, utilization of metabolic substrates, and accumulation of metabolic intermediates and end products, especially during exercise (Wang et al., 1998). On the other hand Ohno et al. (1982) declared that the activity of carbonic anhydrase enzym tended to decrease after the exercise.

The studies that investigate whether L-Carnitine, which plays a key role in lipid metabolism, increases carbonic anhydrase levels are very few. Thus, the aim of the present study was to verify the effects of L-carnitine on carbonic anhydrase level in rats exposed to exhaustive swimming exercise and hyperthermic stress.

MATERIALS AND METHODS

Chemicals

L-carnitine was obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used were analytical grade and obtained from either Sigma-Aldrich or Merck.

Animals and groups

In this study, 24 healthy Spraque Dawley male rats, weighing 250 to 300 g, 4 to 6 months of age, were provided from Fırat University Experimental Animal Research Center (FUDDAM). The study was carried out in Atatürk University Research Center of Experiment Animals and the study was approved by the Ethical Committee of the Atatürk University (AUHADYEK, Ethical Committee Report No: 2008-51). All surgical procedures and protocols used here were in accordance with Guidelines for Ethical Care of Atatürk University Research Center of Experiment Animals.

The rats were kept under special conditions and were sheltered in cages, each with 6 rats, at room temperature (25°C), supplied with food (Bayramoglu Yem Sanayi, Erzurum, Turkey) and water for 12 h day and night cyclus. The rats were divided into four equal groups.

Exercise group 1: Includes rats that underwent exhaustive swimming exercises at a temperature of 38°C.
Exercise group 2 (control group): Includes rats that underwent exhaustive swimming exercises at a temperature of 28°C.
L-carnitine+Exercise group 3: Includes rats that were given L-carnitine and that underwent exhaustive swimming exercises at a temperature of 38°C.
L-carnitine+Exercise group 4 (L-carnitine + control group): Includes rats that were given L-carnitine and that underwent exhaustive swimming exercises at a temperature of 28°C.

In the study, the L-Carnitine was given to the groups 1 to 1.5 h before the exercises in doses of 100 mg/kg by intraperitoneal (I.P.) way.

Exercise protocol

All rats (n: 24) were engaged in fatigue swimming exercises of maximum intensity.

Adaptation training

A swimming pool (80 × 60 ×60 cm^3) was used for 5 min during 5 days at 28°C to ensure the adaptation of rats to the exercises and heat stress. (This temperature is the most appropriate for rat metabolism). A resistance of 2200 V and a digital thermometer (GEMO, micro software and PID thermo controlled device) were used to warm up the pool. After the swimming exercise, the rats were dried with towels, left to rest for 30 min at a warm place and taken back to cages.

Exhaustive training exercise

All groups (n: 24) were swimming at 28 and 38°C until they felt tired. Beginning of uncoordinated actions (inability of floating by

minor extremity actions), remaining under water for 10 s without swimming were determined as tiredness criteria (Osorio, 2003).

Determination of temperatures

American Health Assembly (AHA), approved normal body temperature as 36.5 to 37.2°C. The body temperatures of human beings and rats are the same. A naked person can keep body inner temperature fixed between 12.5 and 55°C in dry weather (Unal, 2002). For the body to feel the heat depends on the temperature of the weather, moisture rate and wind rate. For performance in water sports 26 to 30°C is the optimal temperature (Brooks and Fahey, 1985).

In this study, temperatures of over 38°C were determined as hypertermic, in relation to the optimal temperature for performance (28°C) (Osorio, 2003).

Drawing of blood and preparation of haemolysate

Venous blood was drawn from the V cava inferior into a sterile plastic syringe (10 ml) using a sterile needle. Half of the drawn blood (3 ml) was added to a plastic test tube containing 50 µl of EDTA (1:100) to be used for the carbonic anhydrase enzyme activity assay. Erythrocytes were isolated from fresh rat blood after exhaustive exercise and hyperthermic stress. Immediately, the fresh blood was centrifuged at low-speed centrifugation (1500 rpm) for 15 min (HERMLE Z 323 K) by removal of plasma and buffy coat. The erythrocyte pellet was washed three times with cold 0.16 M KCl and the supernatant discarded. One volume of erythrocyte pellet was suspended in five volumes of ice water to give an erythrocyte haemolysate. CA activity was determined colorimetrically as described above (Coban et al., 2009; Hisar et al., 2005b; Rickli et al., 1964; Wilbur and Anderson, 1976).

Protein determination

Quantitative protein determination was achieved by absorbance measurements at 595 nm according to Bradford's method (1976) previous described (Beydemir et al., 2005; Gulcin et al., 2005c; Koksal and Gulcin, 2008,Senturk et al; 2008), with bovine serum albumin as standard described previously (Sisecioglu et al., 2009; 2010a; 2010b; 2010c; 2010d, 2011).

Hemoglobin estimation

The hemoglobin (Hb) concentration in hemolysate was determined by the cyanmethaemoglobin method. All studies were performed at +4°C (Beydemir et al., 2003; Gulcin et al., 2005d; 2008b; 2009a).

Carbonic anhydrase enzyme assays

Carbonic anhydrase activity was assayed by following the hydration of CO_2 at room temperature according to the method described by Wilbur and Anderson (1976). CO_2-hydratase activity as an enzyme unit (EU) was calculated by using the equation (t_o-t_c/t_c) where t_o and t_c are the times for pH change of the nonenzymatic and the enzymatic reactions, respectively (Beydemir and Gulcin, 2004).

Statistical analysis

The experimental results were performed in triplicate. The data were recorded as mean ± standard deviation and analyzed by SPSS (version 11.5 for Windows 2000, SPSS Inc.). For determining the difference between the mean ranks of two groups, the Mann-Whitney U test which is a non-parametric test ($P<0.01$) regarded as significant, and $P<0.001$, very significant is used.

RESULTS

In this study, the effect of exhaustive swimming exercise and hyperthermic stress on carbonic anhydrase levels in rats was examined. 24 healthy Spraque Dawley male rats divided into four groups. Groups 1 and 2 underwent exhaustive swimming exercise at temperatures of 38 and 28°C (control groups); For Two residuary groups (3 and 4) L- carnitine was given, and mentioned groups underwent exhaustive swimming exercises at the temperature of group 3 at 38°C and Group 4 at 28°C. As can be seen in Figure 1, the results obtained from the present study demonstrated that the least inhibition was observed in the group that was given L-Carnitine and made exhaustive swimming exercises at the temperature of group 3 at 38° C. Results of groups 3 differed significantly from the results of group 4. Results of CA inhibition in group 3 differed significantly from the results of group. CA inhibition of L-Carnitine + Exercise group of 38° C decreased significantly according to Exercise group of 38° C. ($P<0.005$).

DISCUSSION

It is well known that, fatty acid (FA) oxidation increases and stimulates mRNA synthesis of mitochondrial carnitine acyl transferases in skeletal muscle during submaximal exercise. The mechanism is probably related to a combined effect of increased VO_2 max, reduced SNS activity (to some extent an effect of increased VO_2max) and peripheral adaptation to training. The peripheral adaptation in skeletal muscle to endurance training includes increases in mitochondrial content, respiratory capacity, capillary density and lipid oxidation capacity (Mole et al., 1971). The difference in the capacity to oxidize fatty acids (FA) between trained and untrained individuals is due to enhanced cellular FA uptake by the FA-translocase (FAT: CD36) and an increased FA transport into the mitochondria by the L-Carnitine barrier system (Bonen, 1999; Tunstall, 2002).

It is well established that carnitine plays a key role in lipid metabolism. L-carnitine is found ubiquitously in mammalian tissues and represents an important factor in the cellular energy metabolism. Carnitine is essential for the transport of the long-chain fatty acids across the inner mitochondrial membrane into the mitochondrial matrix, the site of β-oxidation (Shimada et al., 2004). L-carnitine prevents oxidative stress and regulates nitric oxide, the cellular respiration (Brown, 1999) and the activity of enzymes involved in defense against oxidative damage (Kremser et al., 1995). Kim et al. (2004) informed that supplementation of L-carnitine and antioxidants may

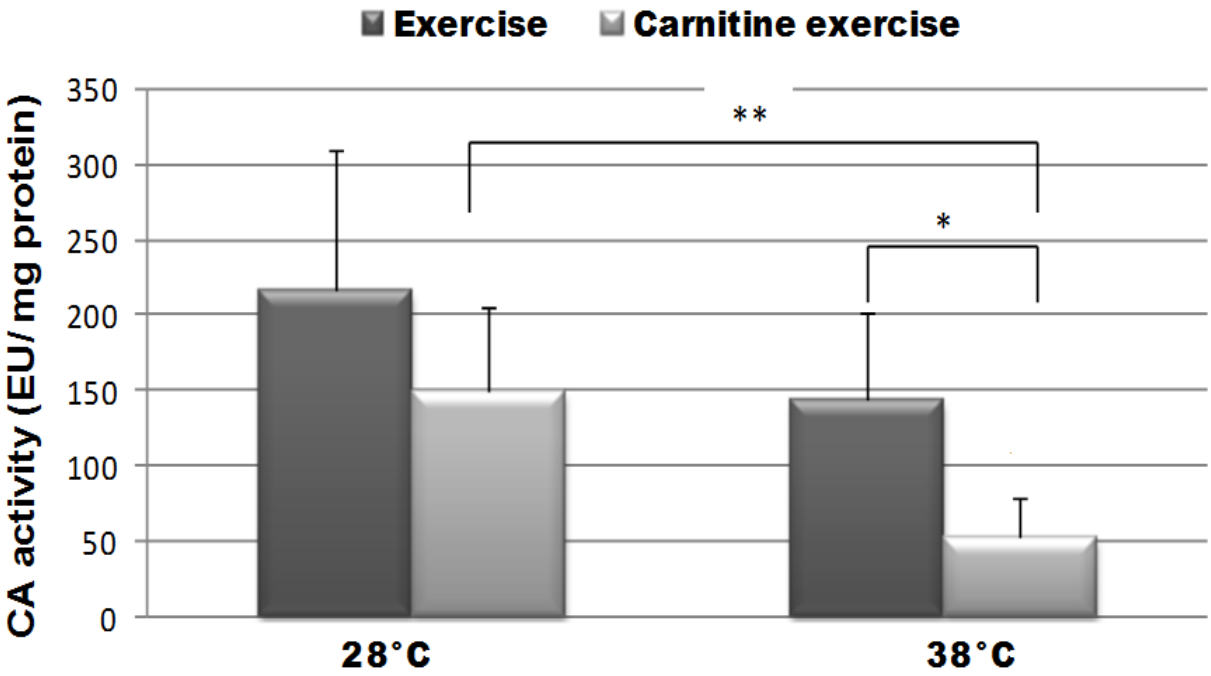

Figure 1. The inhibitor effect of L-carnitine on total carbonic anhydrase levels in rats exposed to exhaustive exercise and hyperthermic stress. Exercise group underwent exhaustive swimming exercise at the temperatures of 38 and 28°C (control group); L-carnitine + Exercise group was given L-Carnitine and underwent exhaustive swimming exercises at the temperatures of 38 and 28°C (L- carnitine + control group). Data are represented by as the means ± standard deviation. There is a significiant difference (**P<0.01) between L-carnitine + Exercise group of 38°C and L-carnitine + Exercise group of 28°C and there is a significant difference (*P<0.005) between L-carnitine + Exercise group of 38°C and Exercise group 38°C.

improve lipid profiles and exercise ability in exercise-trained rats But in some studies, the effect of LC supplementation on aerobic-exercise performance was not reported (Arenas et al., 1994; Huertas et al., 1992)

Carbonic anhydrase (CA) involved in a wide range of physiological and biochemical processes, is present in most tissues including erythrocyte (Hisar et al., 2005b; Senturk et al., 2009). This enzyme is well characterized as a pH regulatory enzyme in many different tissues, and catalyzes reversible hydration of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and H^+ (Gulcin et al., 2004c, 2005d). The CA family consists of thirteen active isozymes in mammals, twelve of which are expressed and function in humans (Hilvo et al, 2005). In many organisms these enzymes are involved in crucial physiological processes connected with respiration and transport of CO_2/bicarbonate, pH, and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (such as gluconeogenesis and lipogenesis), bone resorption, calcification, tumorigenicity, and many other physiologic or pathologic processes (Supuran, 2010). Raisanen et al. (1999) stated that CA III functions as an oxyradical scavenger and thus protects cells from oxidative stress, which has many adverse effects, including lipid peroxidation, protein oxidation and interference with cellular homoeostasis, which can lead to cell death and pathological injury.

When an exercise stress is combined with a high environmental temperature and restricted fluid intake, it seems likely that the adverse effects on cognitive function and subjective feelings will be amplified. These effects may be mediated by reductions in the distribution of the blood volume (Crandall, 2008), leading to reductions in cerebral blood flow (Nybo and Nielsen, 2001) and alterations in regional brain metabolism (Nunneley et al., 2002).These alterations may be sufficient to have detrimental effects on the performance of a number of physiological functions. (Edwards et al., 2007). For exercise intensities associated with a sustained increase in $[La2]_{pl}$ (that is, .V >ET), V CO_2 kinetics become more complex because of additional contributions from aerobic metabolism (associated with a slow component for VO_2), from decreases in muscle and plasma $[HCO_3^-]$ consequent to buffering of lactic acid, and from release of CO_2 from the lung and tissue CO_2 stores by hyperventilation (Wasserman, 1994). Inhibition of carbonic anhydrase (CA) is associated with a lower plasma lactate concentration during fatiguing exercise (Scheuermann et al., 2000). CA inhibition does not impair CO_2 output (VCO_2) at rest or during the steady state of moderate-intensity exercise, but may reduce VCO_2 during maximal exercise. Whereas most studies focus on the

transport and elimination of CO_2 during steady-state conditions, no information is available on the effects of CA inhibition on the kinetics of $V(CO_2)$ in the nonsteady state of whole body exercise in humans (Scheuermann et al., 1999). Tokuda et al. (1984) founded that the rest values of (in erythrocyte carbonic anhydrase) RBC-CA activity were higher in trained subjects (especially in long-distance runners, swimmers etc.), who had undergone long-term strenuous aerobic training than in untrained subjects, and examined that the trained subjects showed higher levels of RBC-CA activity than the untrained subjects before and after training.

It is established that exercise performance is impaired in high ambient temperature (Galloway and Maughan, 1997**).** The body core temperature during exercise varies depending on environmental conditions (Soultanakis, 2003). Many endogenic mechanisms serve in thermoregulation responses (Reilly et al., 2006). During physical exertion an understanding of thermoregulation is important in protecting athletes from injuries and in managing physicial performance under cold and heat conditions. Limited number of studies indicated that L-carnitine, being an antioxidant, has an effect on CA activity at the exercises done under hypothermic and hyperthermic conditions. Şıktar (2009) informed that rats given L-carnitine and underwent exhaustive swimming exercises at the temperature of 18°C (at hypothermic stress condition) demonstrated the highest CA inhibition. According to these results, we may conclude that L-Carnitine does not have a protective effect during exercises done under hyperthermic conditions in rats with reduction of CA activity.

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