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THE EFFECT OF INJECTING ACUTE L-CARNITINE ON ENDURANCE TIME IN RATS EXPOSED TO DIFFERENT WATER TEMPERATURE

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ABSTRACT

Purpose: Carnitine plays an important role in lipid metabolism by transporting long-chain fatty acids into the mitochondria for beta-oxidation. The effect of carnitine on exercise capacity is not clear. The aim of this study was to explain effect of injecting acute L-Carnitine on endurance time in rats exposed to different water temperature.

Material and Methods: Six groups (E18°C, E28°C and E38°C groups made exhaustive swimming CE18°C, CE28°C and CE38°C groups given L-Carnitine and made exhaustive swimming exercises) were formed and a total of 36 Spraque Dawley male rats, weighing 250-300 g were used in this study. In the study, the L-Carnitine was given to the groups 1-1.5 hours before the exercises in the doses of 100 mg/kg by intraperitoneal (I.P.) way. Exhaustive swimming tests were made in a rectangle shaped glass water tank that was 80x60x60 cm³. The uncoordinated movements and staying under the water for 10 seconds without swimming at the surface were accepted as the exhaustion criteria of the rats.

Results: In the rats of CE18°C group the endurance time increased significantly comparing with the E18°C ($P < 0.01$). There was not significantly different among other groups

Conclusion: This result suggests that carnitine may especially enhance the physical performance doing cold ambient. Carnitine might generate that effect by regulation lipid metabolism and mitochondrial functions.

Keywords: L-Carnitine; endurance time; Exercise; Rat; water temperature.

Introduction

Fat and carbohydrates are the primary metabolic fuels utilised by contracting skeletal muscles during exercise and resting (M.J. Watt, J.F. George, Dj. Heigenhauser, Dyck And L.S. Lawrence, 2002). Thus, it is not surprising that a variety of metabolic and biochemical markers is related to exercise performance and training. Manipulations of bioenergetics have frequently been proposed as strategies to enhance exercise endurance or capacity. The functions of carnitine in skeletal muscle are critical to sustaining normal bioenergetics during exercise (P.E. Brass, W.R. Hiatt, 1998).

L-carnitine (4-N-trimethylammonium-3-hydroxybutyric acid), stored within skeletal muscle tissue as either free or acyl carnitine (F.B. Stephens, D.C. Teodosiu, D. Laithwaite, E.J. Simpson, And L. Paul, P.L. Greenhaff, 2006), is an endogenous molecule well-established roles in metabolism (E.P. Brass, 2000), and plays important physiological roles shuttling the long-chain fatty acids across the inner

mitochondrial membrane for ATP production and β -oxidation in peripheral tissues (İ. Gülçin, 2006). For health and performance the importance of mitochondrial function has been highlighted during the last few years (D. Hood, A. Joseph, 2005). It is well known that oxidation of fatty acids (FA) is augmented and lactate formation is reduced during exercise after endurance training. This is explained by an increased mitochondrial density in skeletal muscle and a concomitant increased activity of oxidative enzymes (J. Holloszy, E. Coyle, 1984).

Impairment of muscle contractility due to fatigue may play a role in determining human performance. Through unclear mechanisms, high carnitine concentrations were shown to delay muscle fatigue and permit improved maintenance of contractile force in studies using in vitro animal systems (M. Dubelaar, C. Lucas, W. Hulsmann, 1991; E. Brass, A. Scarrow, L. Ruff, K. Masterson, Van Lunteren, 1993). The relevance of these observations to human exercise is unknown.

Although some researchers declared that L-carnitine supplement, have beneficial effects on exercise performance (F.B. Stephens, D.C. Teodosiu, And P.L., Greenhaff, 2007; J.S. Volek, W.J. Kraemer, M.R. Rubin, A.L. Gomez, N.A. Ratamess, And P. Gaynor, 2002) the others, claimed that taking carnitine before exercise have no effect on performance (C. Greigh, K. Finch, D. Jones, M. Cooper, A. Sargeant, C. Forte, 1987; J.W. Ransone, And R.G. Lefavi, 1997).

Normal body core temperature during exercise varies depending on environmental conditions, such as the situation of training and acclimatization, the duration and intensity of exercise, and individual differences (H.N. Soultanakis-Aligianni, 2003). During physical exertion an understanding of thermoregulation is important in protecting athletes from injuries and in managing physical performance under cold and heat conditions. Thermal stress combined with physical exertion may lead to rise in body core temperature (C.L. Lim, C. Byrne, J.K. Lee, 2008; G.A. Khomenok, A. Hadid, O.P Bloom, R. Yanovich, T. Erlich, O.R. Tal, A. Peled, Y. Epstein, D.S. Moran, 2008). Many endogenic mechanisms serve in thermoregulation responses (T. Reilly, B. Drust and W. Gregson, 2006). However the literature limited knowledge related to the effects of L-carnitine on the exercise done hypothermic and hyperthermic ambient.

The aim of this study was to determine effect of injecting acute L-Carnitine on endurance time in rats made exhaustive swimming exercise in different water temperature, such as 18 °C, 28 °C and 38 °C.

Material and methods

Animals and Groups

In this study, 36 healthy Sprague Dawley male rats, weighing 250–300 g, 4–6 months of age, provided from Firat 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). 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 the room temperature (25°C), supplying with food (Bayramoğlu Yem Sanayi, Erzurum, Turkey) and water for 12-hour day and night cyclus. The rats were divided into six equal groups. Group 1: The ones that made exhaustive swimming exercises at the temperature of 18°C (E18, n=6). Group 2: Group 2: The ones that made exhaustive swimming exercises at the temperature of 28 °C (E28, n=6), Group 3: The ones that performed exhaustive swimming exercises at the temperature of 38 °C (E38 n=6), Group 4: The ones that were given L-Carnitine and made exhaustive swimming exercises at the temperature of 18 °C (CE 18 n=6), Group 5: The ones that are given L-Carnitine and made exhaustive

swimming exercises at the temperature of 28 °C (CE28 n=6), Group 6: The ones that are given L-Carnitine and made exhaustive swimming exercises at the temperature of 38 °C (CE38 n=6).

Chemicals

L-carnitine was obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). In the study, the L-Carnitine was given to the groups 1-1.5 hours before the exercises in the doses of 100 mg/kg by intraperitoneal (I.P.) way (U. Panjwani, L. Thakur, S. Singh, B. Amita, S. Singh, P. Banerjee, 2007).

Exercise Protocol

Maximal intensely tired swimming exercises were applied to exercise and L-Carnitine exercise groups in test group (n: 36).

Adaptation Training. For the rats to have adaptation, they were first made to have swimming exercise in a pool, 80 x 60 x 60 cm³ for 5 minutes during 5 days in water temperature at 26-28°C (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) was used to warm up the pool. After swimming exercise, the rats were dried with towels, made to rest for 30 minutes at a warm place and taken to cages.

Exhausted Swimming Exercise. All the rats in exercise group were made swimming at 18°C, 28°C and 38°C respectively until they felt tired. The uncoordinated movements and staying under the water for 10 seconds without swimming at the surface were accepted as the exhaustion criteria of the rats (R. Osorio, J. Christofani, V. Almeida, I. Picarro 2003).

Determination of temperatures

American Health Assembly (AHA), approved of normal body temperature as 36.5–37.2°C. The body temperature of rats is the same as those of humans. A naked person can keep body inner temperature fixed between 12.5°C and 55°C in dry weather (20). For the body to feel the heat depends on the temperature of the weather, moisture rate and wind rate. 26–30°C is the optimal temperature for performance in water sports (21).

In this study the temperature was determined 10°C more or less than average temperature 28°C as optimal temperature for performance, under 10°C hypothermic (18°C), over 10°C hyperthermic (38°C). In present study to determine temperature values of water, under 16°C and over 38°C posed risk for rats. The rats made to swim at 14 and 39°C died and had severe complications in 5-10 minutes (three out of six).

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.). Differences between exercise and carnitine-exercise group was made using by Mann-Whitney U test. Analysis inside of Group was

made using by Kruskal -Wallis test and $p < 0.05$ was regarded as significant.

Results

Endurance time (minute) between exercise and carnitine-exercise groups, taking place in equal water temperature was indicated in **Table 1**. With reference to, endurance time of CE18 group was significant higher than E18 group ($P < 0.01$). When compared E28 group and CE28 group, CE28 group was obtained a increased, but it is wasn't significantly. Additionally, endurance time wasn't significantly between CE38 group and E38 group.

Table 1. Comparison of endurance time (min.) between exercise and carnitine-exercise groups, taking place in equal water temperature.

Groups	n	mean±sd	Z	P
E18	6	92.00 ± 21.72		
CE18	6	139.00 ± 31.37*	2.722	0.006*
E28	6	249.00 ± 42.46		1.761
CE28	6	291.00 ± 35.16		0.078
E38	6	97.00 ± 39.57		0.962
CE38	6	75.00 ± 39.14	0.336	

(*) $P < 0.01$

Endurance time (min.) among them exercise and carnitine-exercise groups was presented in **Table 2**. With reference to, endurance time of among exercise groups E28 group veiwed significantly a increase in accordance with E18 group and E38 group ($P < 0.05$). If endurance time of among carnitine-exercise groups CE28 group was significant higher than CE18 group and CE38 group ($P < 0.05$). Also, CE38 group was significantly lower than CE18 group ($P < 0.05$).

Table 2. Comparison of endurance time (min.) among them exercise and carnitine-exercise groups .

Groups	Z	P
E28* - E18	-2.201	.028*
E38 - E18	-.420	.674
E38 - E28*	-2.201	.028*
CE28* - CE18	-2.201	.028*
CE38* - CE18	-2.201	.028*
CE38 - CE28*	-2.201	.028*

(*) $P < 0.05$

Discussion

Carnitine is an endogenous compound with well-established functions in cellular metabolism that are clearly important in muscle during exercise (S. Ahmad, H. Robertson, T. Golper, Et Al., 1990). An obligate for optimal mitochondrial fatty acid oxidation,

it is a critical source of energy and also protects the cell from acyl-CoA accretion through the generation of acylcarnitines (E.P. Brass, 2000). Therefore, It is not surprising that the use of supplementary carnitine to improve physical performance has become widespread in recent years.

In this study, in rats given L- Carnitine was evaluate endurance time in exhaustive swimming exercise in different water temperature. According to obtained data, L- Carnitine stimulated endurance time at 18°C of water temperature, although there wasn't sgnificantly in favour of L- Carnitine groups on endurance time between exercise and L-Carnitine exercise groups at 28°C and 38°C (**Table 1**). Also, Comparison of among them exercise groups and L-Carnitine exercise groups was determined effect of L-Carnitine at 18°C (**Table 2**).

In the literature, there were different views with respect to the relationship between exercise performance and L- carnitine. Although there are some theoretical points favouring potential ergogenic effects of carnitine supplementation (O. Heinonen, J. Takala, 1994; H. Karlic, A. Lohninger, 1996), there is currently no scientific basis for healthy individuals or athletes to use carnitine supplementation to improve exercise performance. (O. Heinonen, 1996, C. Greigh, K. Finch D. Jones, M. Cooper, A. Sargeant, C. Forte, 1987; Christoph Stuessi Æ Pierre Hofer Æ Christian Meier Urs Boutellier. L., 2005).

Slipandi (N. Silprandi, F. Dilisa, G. Pteralisi, P. Ripari, F. Maccari, R. Menabo, Ma. Giamberardino, 1990), and Vecchiet(L. Vecchiet, F. Di Lisa, G. Pteralisi, P. Ripari, R. Menabo, M. Giamberardino, N. Silprandi, 1990) noted that ingested 2 g. L-Carnitine before 60 min. exercise decreased blood lactat and increased performance and strenght. Greig et al.(C. Greigh, K. Finch D. Jones, M. Cooper, A. Sargeant, C. Forte, 1987) declared that carnitine supplementation wasn't beneficial effect to exercise performance since the observed effects were small and inconsistent.

The reserches relevant to effect of L- Carnitine on endurance time in the literature are limited number. Likewise, Trappe et. al. (S. Trappe, D. Costill, B. Goodpaster, M. Vukovich, W. Fink, 1994), notified that L-carnitine supplementation does not provide an ergogenic benefit in performance times in highly trained swimmers. Another study making on thirty-two male rats was pointed out that in exercise endurance time were no changes by supplementation in untrained animals, however endurance times were longer in long-trained supplemented animals than in long-trained non-supplemented.(E. Kim, H. Park, Y. Cha, 2004).

The thermoregulatory mechanisms play important roles in maintaining physiological homeostasis during rest and physical exercise. Physical exertion poses a challenge to thermoregulation by causing a substantial increase in metabolic heat production. However, within a non-thermolytic range,

the thermoregulatory mechanisms are capable of adapting to sustain physiological functions under these conditions (L. Chin Leong, C. Byrne, J. Lee, 2008).

Many endogenous mechanisms serve in thermoregulation responses (T. Reilly, B. Drust, And W. Gregson, 2006). However the literature knowledge related to the effects of L- carnitine on the exercise done hot and cold ambient. Jansens et al., (G.P.C. Janssens, J. Buyse, M. Seynaeve, E. Decuypere, And R.D. Wilde, 1998), announced that heat production has decreased in exercising pigeons after L-carnitine supplementation.

Skeleton muscles have used free fatty acids both at rest and during exercise. For this reason, L- Carnitine deficiency may cause to decrease in skeleton muscle functions and in exercise capacity, In humans, cold-induced thermogenesis is attributable to skeletal muscle contractile activity (U. Chu, M. Larsson, T. Moen, S. Rennard And L. Bjermer). Humans initiate this thermogenesis through involuntary shivering or by voluntarily modifying behavior, i.e., increasing physical activity . While certain animals exhibit an increased metabolic heat production by noncontracting tissue (brown adipose tissue) in response to cold exposure. In cold ambient, in skeleton muscles increase using free fatty acids both at rest and during exercise (A. Strup, 1986; B. Cannon And J. Nedergaard, 2004). For this reason, L- Carnitine deficiency may cause to decrease in skeleton muscle functions and in exercise capacity (S. Gültük, A. Demirkazık, S. Erdal, T. Demir 2007). Exercise intolerance, carnitine palmitoyl-transferase enzyme deficiency (CPT II) has been postulated to depend on low - carbohydrate-high - fat diet, exhaustive exercise, fasting, hypothermia and insomnia (M. Orngreen, R. Ejstrup, J. Vissing, 2003), and especially, it created skeletal muscle damage (A. Gentili, E. Lannella, F. Masciopinto, M. Latrofa, L. Giuntoli, S. Baroncini, 2008). Little is known about energy substrate metabolism and energy utilization in hibernating species under conditions of hypothermia and rewarming. Belke et al (D. Belke, L. Wang, G. Lopaschuk, 1997), reported that total energy substrate metabolic rates were greater in rat than ground squirrel hearts during hypothermia, despite a lower level of work being performed by the rat hearts, indicating that rat hearts are less efficient than ground squirrel hearts during hypothermia. Because of this reasons, in cold water - in view of improved heat production and energy metabolism- increased using fatty acids. this study may say that L- Carnitine supplement helped to energy output at 18°C water temperature.

Conclusions

In conclusion, according to this study carnitine may especially enhance the physical performance doing cold ambient. Carnitine might generate that effect by regulation lipid metabolism and mitochondrial functions. Effect of this study Carnitine

will inform useful that to be supported by molecular trial and advanced researches

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SOME DIFFERENCES IN PARAMETERS OF BONE MINERAL METABOLISM IN VARIOUS SPORT BRANCHES

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ABSTRACT

Objective

This study was carried out in order to compare the differences of laboratory parameters related to bone metabolism such as alkaline phosphatase (ALP), serum calcium (Ca), magnesium (Mg) and phosphorus (P), in various sport branches.

Material and Method

Serum alkaline phosphatase, calcium and phosphorus, and magnesium levels were measured in 23 skiers, 21 runners, 24 wrestlers, 20 handball, 21 soccers and 30 sedentary living healthy individual.

Results

The groups were matched according to age and sex. As expected, there were no significant differences according to age or the female-male ratio between the athletes and controls subjects ($p>0.05$).

Serum alkaline phosphatase (ALP), serum calcium (Ca), magnesium (Mg) and phosphorus (P) were determined in the athletes and the healthy control subjects. In all the athletes and controls, routine biochemical parameters including alkaline phosphatase (ALP), serum calcium (Ca), magnesium (Mg) and phosphorus (P) were within normal limits (**Table 1**). The wrestlers had lower levels of calcium compared to control subjects ($p<0,05$). The runners and handball had higher levels of phosphatase compared to control subjects ($P<0,05$). Other parameters had no significant difference between athletes and controls (Table 2).

Conclusion

When compared with control group, it has been found that wrestlers have low level of CA while runners and handball players have high level of P.

Key words: Serum alkaline phosphatase, calcium, magnesium, phosphorus and athletes.

Introduction

Serum alkaline phosphatase is a member of a family of zinc metalloprotein enzymes that function to split off a terminal phosphate group from an organic phosphate ester. This enzyme functions in an alkaline environment (optimum pH of 10). Alkaline Phosphatases are a group of enzymes found primarily the liver (isoenzyme ALP-1) and bone (isoenzyme

ALP-2). The primary importance of measuring alkaline phosphatase is to check the possibility of bone disease or liver disease. For an adult, 50-75 mg/dl is considered a reasonable optimal range (O. Maldonado, R. Demasi, Y.Maldonado et al. 1998, N. McIntyre, S. Rosalki, 1991, AG,Lieverse, GG. van Essen, GJ, Beukeveld. et al. 1990). Calcium is the basic mineral component of the skeleton and plays major roles in neurologic