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EFFECT OF L- CARNITIN AND THERMAL STRESS ON FREE RADICAL AND ANTIOXIDANT LEVELS IN RATS DURING THE EXHAUSTIVE SWIMMING EXERCISES AT HYPOTHERMIC AND HYPERTHERMIC WATER TEMPERATURES

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SUMMARY

Carnitine plays an important role in lipid metabolism by transporting long-chain fatty acids into the mitochondria for beta-oxidation.The aim of this study was to explain the effect of acute L-carnitine on antioxidant levels and lipid peroxidation in rats exposed to thermal stress.

Eight groups (E18°C, E28°C and E38°C exhaustive swimming groups; CE18°C, CE28°C and CE38°C L-carnitine and exhaustive swimming exercises groups; CS28°C (the sedentary group which is given carnitine); S28°C (only the sedentary group), were formed and a total of 48 Sprague 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 cm^3 in size. Uncoordinated movements and staying under the water for 10 seconds without swimming at the surface were accepted as the exhaustion criteria of the rats. In the blood samples, the SOD, GR, POD and CAT are analyzed with spectrophotometric method, and MDA is analyzed with HPLC-FLD method. In the statistical analysis of the study, Anova, Duncan and T Tests are used.

According to the results of the study, the MDA level of the CE18°C group is significantly higher than those of CE28°C and CE38°C groups ($p<0.05$). While the GR levels of E18°C group is significantly higher than the CE18°C group ($p<0.05$,), the POD levels are significantly low ($p<0.05$,). The POD levels of CE28°C group is significantly higher than those of E28°C group ($p<0.05$).

The findings that are gathered at the end of the study showed that the oxidative stress increases under hypothermic circumstances and it cannot prevent the free radical formation of the L-carnitine under hypothermic conditions, in addition it increases the antioxidant activity under thermal stress. The L-carnitine supply under thermal stress may show an antioxidant effect and be useful in sports activities.

Keywords: L-carnitine; exercises; rat, water temperatures, antioxidant enzymes; lipid peroxidation.

1. Introduction

The adaptation in skeletal muscle to endurance training includes increases in mitochondrial content, respiratory capacity, capillary density and lipid oxidation capacity (K.Sahlin, RC. Harris, 2008). L-carnitine plays a key role in lipid metabolism (M.S.Aoki., Almeida ALRA., F.Navarro, LFBP. Costa-Rosa., RFP.Bacurau, 2004). L-carnitine (4-*N*-trimethyylammonium-3-hydroxybutyric acid), stored within skeletal muscle tissue (E.Broad, C. Bolger, S.Galloway, 2006) and cardiac muscle as either free or acyl carnitine (FB. Stephens, DC.Teodosiu, D. Laithwaite, E.J. Simpson, L. Paul, P.L.Greenhaff, 2006), 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 (I.Gülçin, 2006).

It is still a subject of discuss whether the administration of L-carnitine improves performance of intensive endurance exercise (E.P.Brass, 2000). Several researchers indicated that L-carnitine supplement, have benefitial effects on exercise performance and thus it increases fat oxidation during prolonged exercise, preserves glycogen stores and delays the onset of fatigue (F.B.Stephens, D.C. Teodosiu, and P.L. Greenhaff, 2007, J.S. Volek, W.J.Kraemer, M.R. Rubın, A.L. Gomez, N.A. Ratamess, and Gaynor, 2002, C. Greigh K.M.Finch, D.A.Jones, M. Cooper, A.J.Sargeant 1987), clained that in researches they carried out with various different exercise, taking carnitine before exercise or increasing of acute carnitine have no effect on performance, and , Ransone et al., L- carnitine has no effect on the lactic asid accumulation in max. anaerobic exercises.

The body core temperature during exercise varies depending on enviromental conditions, such as the situation of training and acclimatization, the duration and intensityof exercise, and individual differences (Soultanakis-Aligianni, H.N.). During physicial exertion an understanding of thermoregulation is important in protecting athletes from injuries and in managing physicial performance under cold and heat conditions.

Thermal stress combined with physical exertion may lead to rise in bady 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 responces (Reilly, T., Drust, B., and Gregson, W., 2006). However the literature knowledge related to the effects of L-carnitine on the exercise done hypothermic and

hyperthermic ambient. (G.P.C.Janssens, J. Buyse, M.Seynaeve, E.Decuypere, R.D.Wılde, R.D., 1998), announced that heat production has decreased in exercising pigeons after L-carnitine supplementation. Exercise intolerance carnitine palmitoyl-transferase enzyme deficiency (CPT II) has been postulated to depend on low-carbohitrate-high-fat diet, exhaustive exercise, fasting, hypothermia and insomnia (M.C.Orngreen, R.Ejstrup, J.Vissing, 2003), and especially, it created skeletal muscle damage (A.Gentili E.Lannella, F.Masciopinto, M.E.Latrofa, L.Giuntoli, S.Baroncini , 2008).

In this study, we researched the effect of acute L-carnitine on antioxidant levels and lipid peroxidation in rats exposed to thermal stress. L-carnitine is an antioxidant, and it prevent accumulating end product of lipid oxidation (I. Gülçin, 2006).

2. Material and methods

2.1. Chemicals

L-carnitine, Nitroblue tetrazolium (NBT), xanthine, xanthine oxidase, oxidized glutathione,tiobarbituric acid (TBA), NADH and ABTS were obtained from Sigma company (Sigma-Aldrich GmbH, Sternheim, Germany). 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.

2.2. Animals and groups

In this study, 48 healthy Sprague Dawley male rats, weighing 250–300 g, 4–6 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 of which has 6 rats, at the room temperature (25°C). The food (Bayramoğlu Yem Sanayi, Erzurum, Turkey) and water were supplied for 12–hour day and night cycles. 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.

E (exercises, 18°C): The exhausted swimming exercises group at the temperature of 18°C).

E (exercises, 28°C): The exhausted swimming exercises group at the temperature of 28°C

E (exercises, 38°C): The exhausted swimming exercises group at the temperature of 38°C.

CE (L-carnitine-exercises, 18°C): The exhausted swimming exercises group with L-carnitine at pharmacological dosage (100 mg/kg) at the temperature of 18°C.

CE (L-carnitine-exercises, 28°C): The exhausted swimming exercises group with L-carnitine at pharmacological dosage (100 mg/kg) at the temperature of 28°C.

CE (L-carnitine-exercises, 38°C): The exhausted swimming exercises group with L-carnitine at pharmacological dosage (100 mg/kg) at the temperature of 38°C.

S (sedentary, 28°C): at 28°C, the sedentary group which no any practise (the control group).

CS (L-carnitine-sedentary, 28°C): The sedentary group at the temperature of 28°C with L-carnitine at pharmacological dosage (100 mg/kg) (the control group)

Exercise Protocol

Maximal intensely exhausted swimming exercises were applied to exercise and L-carnitine exercise groups in test group

Adaptation Training: For the rats to have adaptation, they were first made to have swimming exercise in a pool, 80 x 60 x 60 cm^3 for 5 minutes at 28ºC during 5 days (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 swimming exercise, the rats were dried with towels, made to rest for 30 minutes at a warm place and taken to cages.

Training: Training of Maximal Exhaustive Swimming Exercise. The exercise group (n:36) were made to swim at 18ºC, 28ºC and 38ºC until they felt tired. Beginning uncoordinated actions (unability of floating by minor extremity actions), remaining under water for 10 seconds without swimming were determined as tiredness criteria (RAL, Osorio, J.S. Christofani, VD. Almeida, AK.Russo, IC.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 (E. Siktar). For the body to feel the heat depends on the temperature of the weather, moisture rate and wind rate. 26–30ºC is the optimal temperature for performance in water sports (G.A.Brooks, T.D.Fahey, 1985).

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

Drawing of blood

Venous blood was drawn from the vein portae into a sterile plastic syringe (10 mL) using a sterile needle. Half of the drawn blood (5 mL) was added to a

plastic test tube containing 50 µL of heparin (1:100) to be used for the antioxidant enzymes assay, and the other 5 mL of drawn blood was added to a glass vacationer containing 15% EDTA (0.054 mL/4.5 mL) to be used for MDA levels.

2.4. Preparation of Hemolysate

Erythrocytes were isolated from fresh rat blood, which was obtained from the University Hospital Blood Centre following low-speed centrifugation at 1500 rpm for 15 min (MSE, MISTRAL 2000) by removal of plasma and buffy coat. The red cells were washed twice with 0.9% w/v NaCl and hemolyzed with 1.5 volumes of ice-cold water. Ghost and intact cells were then removed by high speed centrifugation at 20000 rpm for 30 min (Heraeus Sepatech, Suprafuge 22) at 4°C.

2.5. Hemoglobin Estimation

The hemoglobin (Hb) concentration in hemolysate was determined by the cyanmethaemoglobin method. All studies were performed at +4°C (Olcay H, Beydemir Ş, Gülçin İ, et al. Supuran, C.T, 2005).

2.6. Antioxidant Enzymes Activity Assays

SOD activity

SOD activity is one of the most important indicators of tissue antioxidant capacity. SOD activity determination was performed in the samples before and after adding trichloroacetic acid (TCA, 20%), as described. In this method, xanthine-xanthine oxidase complex produces superoxide radicals that react with nitroblue tetrazolium (NBT) to form a farmazane (NBT^{2+}) compound. SOD activity is measured at 560 nm by detecting inhibition of this reaction. By using blank reactions in which all reagents are present except the supernatant sample and by determining the absorbance of the sample and blank, SOD activity is calculated (Sun Y, Oberley L, Li Y, 1988).

Catalase activity

Catalase activity was assayed by monitoring the decrease in absorbance at 240 nm due to H_2O_2 consumption according to the method of (Aebi H., 1984). The reaction mixture contained 25 µL hemolysate, 0.5 mL 10 mM H_2O_2 and 0.9 mL 30 mM potassium phosphate buffer (pH 7.3). The decrease in absorbance was recorded at 240 nm for 60 sec. Enzyme activity was expressed as µmoles H_2O_2 decomposed by using the H_2O_2 extinction coefficient 36 $\mu M^{-1}cm^{-1}$.

Peroxidase activity

Peroxidase activity was measured by the method of (Shannon LM, Kay E, Law JY., 1966). For this purpose, 0.1 mL hemolysate was taken up into 1.7 mL 0.05 M potassium phosphate buffer (pH 7.3). Then 0.5 mL ABTS was added. The reaction was started by addition of 0.2 mL 0.2 M H2O2. Change in absorbance at 470 nm was recorded for 2 min at intervals of 15 sec. The enzyme activity was expressed as enzyme unit.$min^{-1}gHb^{-1}$.

Glutathione Reductase Activity

Glutathione reductase activity was estimated by the method of (Goldberg DM, Spooner RJ., 1983). To 0.1 mL haemolysate 2.5 mL 120 mM potassium phosphate buffer (pH 7.3), 0.1 mL 0.015 mM EDTA and 0.1 mL 0.065 mM oxidised glutathione were added. After 5 min, 0.05 mL 9.6 mM NADH were added and mixed. The absorbance was recorded at 340 nm at intervals of 15 sec. The enzyme activity was expressed as nmole NADH oxidised min–1.gHb–1 protein using the molar extinction coefficient 6.23 µM–1cm–1.

Determination of MDA

MDA concentrations in blood plasma samples were measured by the high-performance liquid chromatography with fluorescent detection (HPLC-FLD) previously described . Briefly 50µL of plasma sample was mixed in (0.44 M) H_3PO_4 and (42 mM) tiobarbituric acid (TBA), and incubated for 30 min in a boiling water bath. After rapidly cooling on ice, an equal volume alkalen methanol was added to the sample, votex, centrifuged (3000 rpm for 3 min), and the aquous layer was removed.

Then, 20 µL supernatant was analyzed by HPLC (HP, Agilent 1100 moduler systems with FLD detector, Germany): Column, RP-C18 (5 µm , 4.6 x 150 mm , Eclipse VDB- C18. Agilent) elution, methanol (40:60, v/v) containing 50 mM KH_2PO_4 buffer (pH 6.8) flow rate 0.8 mL/min. . Fluorometric detection was performed with excitation at 527 nm an emission at 551 nm. The peak of MDA-TBA adduct was calibrated a TEP (1,1,3,3- tetraethoxypropane) standart solition procedded in exactly the same as plasma and urine sample (Khoschsorur GA, Winklhofer-Roob BM, Rabp H, 2000).

2.7. 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.). One-way analysis of variance ANOVA was performed by procedures. Significant differences between means were determined by Duncan's Multiple Range tests, and $p<0.05$ was regarded as significant, and $p<0.01$ was very significant.

3. Results

According to effect of L-carnitine, there was no difference between the groups took place the same water temperature (as E18°C and CE18°C) with regard to levels of MDA in Fig.1. The SOD values for serum increased significantly in the CE 38°C group according to the E 38°C group ($P<0.05$).The mean of standard derivations of groups respectively, 6.96±0.88 and 10.02±2.08. There was no difference between the E 18°C group and the CE 18°C group, between the E 28°C group and the CE 28°C group and between the S 28°C group and the CS 28°C group in Fig.2.

In Fig. 3, GR levels decreased significantly the CE 18°C than the E 18ºC group ($P<0.05$). The values of CE 18°C and the E 18ºC group were found as 0.31± 0.07 and 0.21±0.07. Not significant between E 28°C and CE 28°C, E 38°C and CE 38°C and S 28°C and CS 28°C. While it was determined a significant increase in

the levels of POD the CE 18°C group compared with the E 18°C group and the CE 28°C group compared with the E 28°C group *(P*<0.05), was decreased significantly the S 28°C group according to the CS 28°C group (p< 0.05).The mean of standard derivations of groups respectively, 2.97±1.20 and 4.45±0.75; 2,87 ± 0,57 and 3,77 ± 0,32; 2.37±0.94 and 4.03±0.58) in Fig 4. The CAT values were not significant in any groups in the same temperature in Fig 5.

According to the effect of thermal stress, the levels of MDA, while there were no different significantly among exercise groups, among L-carnitine-exercise groups, the levels of MDA of CE 18°C group increased more than the CE28°C and the CE38°C group. SOD levels decreased a significantly the E 38°C group compared with the E 18°C group and the E 28°C group (p< 0.01). GR levels, the E18°C and the E38°C group increased significantly according to the E 28°C group (p< 0.05). CAT levels are not significant both exercise groups and L-carnitine-exercise groups in different temperature in Tab.1.

4. Discussion

In this study was aimed that at the rats are made acute swimming exercises at different water temperatures is determined the effect of carnitine and thermal stress on the free radical accurance and antioxidant, no different significant in the L-carnitine-exercise and exercise groups took part in the same temperature at serum MDA level (fig 1). The SOD levels of 38°C L- carnitine exercise group is significant than 38°C exercise group (P<0.05) (fig. 2). GR levels decreased significantly the CE 18°C than the E 18ºC group (*P*<0.05) (fig. 3).

The POD levels of 28°C L-carnitine exercise group was higher than 28°C exercise group (P<0.05). The POD levels of 28°C L-carnitine- sedentary group increased significantly compared with the 28°C sedentary group (fig.4).

L-carnitine is a essential subject which supplies transportation of fat acids long-chain from stoplasma to mitochondria that is known as energy stocks for cells (C.J. Reouche, 1996, W.O.Hulsmann, A.Peschechera E.Martelli, 1994). It increases the general metabolic activity (T.M.Hagen, J.Liu, J.Lykkesfeldt, C.M.Wehr, T.Russell, I.V.Vinarsky, J.C.Bartholomew, 2002) by developing mitochondrial functions. Besides, L-carnitine prevents oxidative stress, the cellular respiratory and enzym activities protects versus oxidative damage, and regulate the nitric oxidation (D.S. Sachan, N.Hongu, M. Johnsen., 2009). L-carnitine is an antioxidant, and it prevents accumulating end product of lipid oxidation (Gülçin, İ., 2006). It is a tricarbondialdehid which has been produced by the dissuasion of multiple unsaturated fat acids being reacted to malondialdehid (MDA) peroxidation which is the lost combination of lipid peroxidation (B.E.Kurutaş, G.F.İnanç M.Kılınç 2004, G. Cighetti, L.Duca, L. Bortone, S. Sala, I.Nava, G.Fiorelli, M.D.Cappellini, 2002). (K. Karanth, K. Jeevaratnam, 2005) recorded that it helped the lipid peroxdation and prepared protection against oxidative destructions regulating the distribution of blood in L-carnitine supplement exercise and the GSH in the muscles, blood and liver at the trained rats in a period of six montly. (P.Rajasekar, CV.Anuradha, 2007). Found out that the external L-carnitine increased the insulin sensivity and decreased lipogliko toksitite and oxidative destruction in the skeleton muscles at the rats which are fed by fruktoz in their studies (J.S.Volek, W.J.Kraemer, M.R.Rubın, A.L.Gomez,N.A. Ratamess, and P.Gaynor, 2002) reported in their studies that the serum MDA level significantly increased after the resistance exercises to the while body, the MDA level reached the pre-exercises level in 15 minutes in the group where carnitine was realized and the MDA level decreased to the normal values in 180 min in the placebo group.

Being different from these studies the acute exercise may be the reason of so much influence of L-carnitine on MDA. Because, numerous studies in literature were stated that the realization of L-carnitine was not put given any positive result so for during acute exercise. (P. Colombani, C.Wenk, I.Kunz, S. Krahenbuhl, M.Kuhnt, M. Arnold, P.Frey-Rındova, W. Frey, W.Langhans, 1996) determined that acute L-carnitine was not raised the running performance before and during long-run (20km. marathon). (Nuesch R, Rosetto M, Martına B., 1999) reported that 1g. of L-carnitine that was taken before and after the exercise made at the treadmill didn't improve the maximum exercise performance as well.

In our study the parameters of SOD, GR, POD and CAT were determined as the sign of the antioxidant system. While at the exercise group raised the GR level at hypothermic condition, at the L-carnitine groups increased SOD and POD levels. It can be said that L-carnitine is only influential on POD and SOD. In the some studies, L-carnitine was defined as an antioxidant which prevents the gathering of the last production of lipid oxidation (Fabriello RG, Calabrese F., 1988).

Namely, In the other study it was reported that L- carnitinee was turned the changes happend in the brain at old rats upside down, as an antioxidant (S. Kumaran, B.Deepak, B.Naveen, C.Panneerselvam 2003). Findings in another study stated that the supplements of L-carnitine (2g/ day) has reduced free radical occurrence and tissue destruction and the purine production before and after the mild squat exercise (J.S.Volek, W.J. Kraemer, M.R.Rubın, A.L.Gomez, N.A. Ratamess and P. Gaynor, 2002). L-carnitine has also protective influence on the 3-nitropropionic acid (3-NPA) induced neurotoxicity which increased by hypothermia (Z.C.Binienda, S.F.Ali, 2001). Although the studies which show the influence of L-carnitine related with hot and cold environment are few, the findings of this study support the findings of other studies. So, in this study, it can be said that the hypothermic conditions have given on additional lead to the metabolism in the CE 18ºC group and it

increased the use of fat acids during acute aerobic swimming exercises.

The increase of the POD level in advance of the L-carnitine groups in the sedanter groups may result the influence of L-carnitine antioxidant. (Sachan DS, Hongu N, Johnsen M., 2005) reported that choline and carnitine supplementation reduced oxidative stress, and the mild exercise regimen was not a deterrent to this outcome in humans. (J.D.Robertson, R.J.Maughan, G.G.Duthie, P.C.Morrice,1991) found out that the MDA values are higher in sedentary after the acute exercises in their study they made on twenty runners who make six sedentary and different exercises. In another study, it was stated that the increase of GSH, GSSD is related with LDL hydroperoxides and MDA decreases and 8-epi PGF_2 α decreases (L.Tesoriere, D.Butera, A.M.Pintaudi, M.Allegra, M.A.Livrea, 2004). These findings seem to be similar to our study.

On the other hand, at the rats swimming at 18°C, 28°C and 38°C, in terms of thermal stress influence, the MDA levels did not shown significantly differences among exercise groups (18,28, 38°C). It increased other much more in the 18°C L-carnitine-exercise than in the 28 and 38°C group ($P<0.05$). There is not so much differences at the SOD levels in the 18°C and 28°C exercise groups where as both groups are quite bigger in terms of SOD levels than the 38°C exercise groups ($P<0.01$). The GR levels in the 28°C exercise group reduced much more than the 18°C-38°C groups($P<0.05$). Although in the 18°C-28°C groups there is only a slight difference among the POD levels, the POD levels have been recognized quite high between these two groups the 38°C exercise group ($P<0.05$, table 1). Due to thermal temperature and ischemi-reperfuzyon tissue destruction may happen during sportive activities. Scientists of sports have explained the reason of the ROT production took place during the sportive activities with the chain of mitokondriyal electron transfer, the ksantin oxidaz system, reactions with metal catalizör and active nötrofillers (J.Peake, K.Suzuki, 2004). Although the reactive oxsygen types (G.Cohen, R.Heikkila., 1974) have been related with some physiology- biochemical events such as O_2 consumption, lactic acid production (H.B.Demopoulos, J.P.Santomier, M.L.Seligman, 1986) and hyperthermia (T.Matsuzuka, N.Sakamoto M.Ozawa, A.Ushitani, M.Hirabayash, Y.Kanai, 2005) , in literature, the relation between them have not been completely yet (L.L. Ji, R.G. Fu, E.W. Mitchell., 1992).

The literary knowledge related with the influence of L-carnitine on the exercises made in hypothermic and hyperthermic condition is limited. There is a few information about the regulation of energy metabolism and cardiac function of the hibernating animals in hipotermic conditions. Darel and et al. reported that the energy matabolism increases in the rat heart during hypotermia regardless off cardiac work on their study (D.D.Belke, L.C.H. Wang, G.D.Lopaschuk, 1997). Events such as diet with low carbohydrate, high fat, long exercise, starving, extreme cold and insufficient sleeping lead to deformation especially in sceleton muscules increasing fat acid oxidation and causing carnitinee palmitoyl-transferase enzyme deficiency (CPT II) (A. Gentili, E. Lannella, F. Masciopinto, ME. Latrofa, L. Giuntoli, S. Baroncini., 2008). The effect of carnitine supplement changes depending on the duration and type of exercise, generate of the experiment, duration and amount of dose, and its permenant steady use (M. Shafi, 1998). Although the amount of L-carnitine we have used is enough under normal exercise conditions, it may be insufficient to reduce the MDA level in the negative conditions of hipotermic environment.

In literature there are studies of the same opinion that hypothermic conditoin created more stress . Osorio et al.emphasized that they reached the highest TBARS at 39°C in thermal stress situation in their study on pregnant rats (R.A.L. Osorio, J.S. Christofani, V.D. Almeida, A.K. Russo, I.C. Picarro., 2003). In rats, reactive oxygen types increase heart, skeleton muscle, plasma during exercise, and it stimulates the antioxidant mechanism (S. Kumaran, B. Deepak, B. Naveen, C. Panneerselvam, 2003). It is an important reactor against thermal depression antioxidant defence mechanism on trained animals (R.A.L. Osorio, J.S. Christofani, V.D. Almeida, A.K. Russo, I.C. Picarro, 2003). In this study the difference of SOD, POD and GR levels among the heat groups proves it.

(R.A.L. Osorio, J.S. Christofani, V.D. Almeida, A.K. Russo, I.C. Picarro, 2003). Examined that the metabolic response to exercise doesn't change under thermal stress on pregnant rats. Furthermore, they recommended avoiding hot water as it would be dangerous for the baby when the mother is under extreme hot and cold stress and would be hazardous for the fatal development at water temperature while swimming although the metabolic responses continue. In this study, the ineffectiveness of temperature differences in L-carnitine-exercise groups in terms of antioxidant levels show that the effects of L-carnitine increase in hypothermic and hyperthermic situations. In a study it has been claimed that when met with standard antioxidants which are natural antioxidant such as α-tocoferol, the L-carnitine activities are effective antioxidants in strength decreasing of different in vitro evaluations such as combination with metals, cleaning of hydrojen peroxide, removing superoxide anyone and DPPH radicals (I.Gülçin, 2006).

Consequently, in this study in which the effect of carnitine has been researched according to different water temperatures, it has been thought that, the significantly increase of the MDA level in hypotermic situation in the carnitine group according to the effect of thermal stress, is related with the additional load on metabolism caused by hypotermic conditions and the increase in utilization the fat acids during swimming exercises. The increase of SOD, POD and CAT levels in favour of L-carnitine between the sedanter group and L-carnitine sedanter group reflects the positive effect of L-carnitine on oxidative stress without being

under any stress (heat or exercise). Providing additional carnitine in increasing the antioxidant levels in the organism especially against the harmful effects us oxidative stress under thermal stress may be useful for athletes. However, It has been considered that it would be useful in experiencing this practive in both in the vitro and in the vivo studies in long duration endurance exercise under thermal stress. It can be taken into consideration that the acute exercise may be restrictively useful to provide a clear result under thermal situation.

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Table1: Effect of thermal stress on values of MDA, SOD, GR, POD and CAT in exercise and L-Carnitine -exercise groups at different water temperature.

		Exercise Groups		L-Carnitine–Exercise Groups	
Parameter	**Temperature**	**Mean ±S.D.**	**P**	Mean ±S.D.	P
MDA (nmol/ml)	18°C	0,42 ± 0,24	**0.402**	0,43 ± 0,10 [b]	**0.019***
	28°C	0,32 ± 0,17		0,26 ± 0,09 [a]	
	38°C	0,27 ± 0,07		0,23 ± 0,13 [a]	
SOD (EU/ml)	18°C	13,08 ± 3,54 [a]	**0.002****	10,61 ± 1,41	**0.947**
	28°C	10,18 ± 1,40 [a]		10,66 ± 2,90	
	38°C	6,96 ± 0,88 [b]		10,2 7± 2,04	
GR (Eu/gHb)	18°C	0,31 ± 0,07 [a]	**0.012***	0,21 ± 0,07	**0.146**
	28°C	0,17 ± 0,09 [b]		0,21 ± 0,03	
	38°C	0,28 ± 0,04 [a]		0,27 ± 0,05	
POD (EU/gHb)	18°C	2,97 ± 1,20 [b]	**0.012***	4,45 ± 0,75	**0.338**
	28°C	2,87 ± 0,57 [b]		3,77 ± 0,32	
	38°C	4,57 ± 1,26 [a]		4,23 ± 1,09	
CAT (Eu/gHb)	18°C	282,24±71,32	**0.519**	293,72±58,67	**0.949**
	28°C	241,06±81,08		276,58±88,67	
	38°C	311,86±146,57		280,20±128,29	

*P<0.05: significantly difference beetwen a>b **P<0.01(in exercise groups) Significantly difference beetwen a>b (in exercise groups). ***P<0.05: significantly difference beetwen b>a (in L-Carnitine and exercise groups).

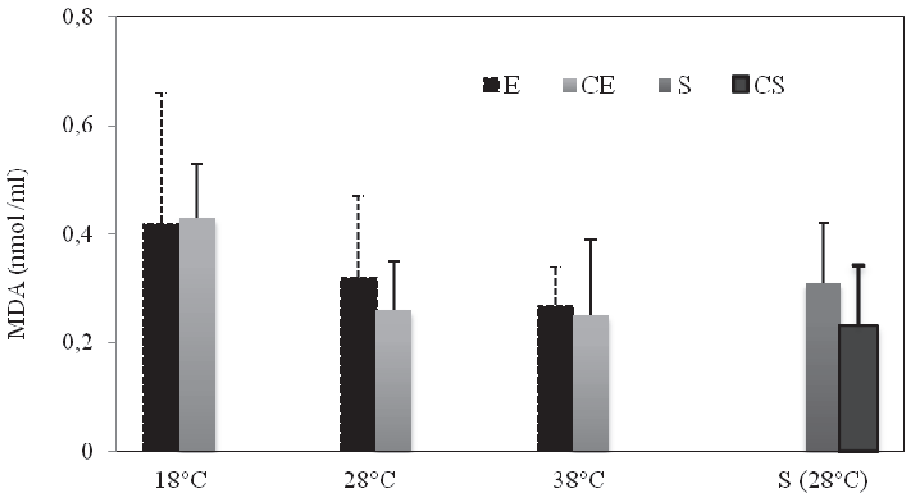

Figure 1.

Figure 1. Effect of L-carnitine on **MDA** levels in exercise and L-carnitine-exercise groups situated the same temperature. **E**, exercise groups; **CE**, L-carnitine-exercise groups; **S**, sedentary groups; **CS**, L-carnitine-sedentary groups. No significant differences between the groups took place in the same water temperature (*P*>0.05).

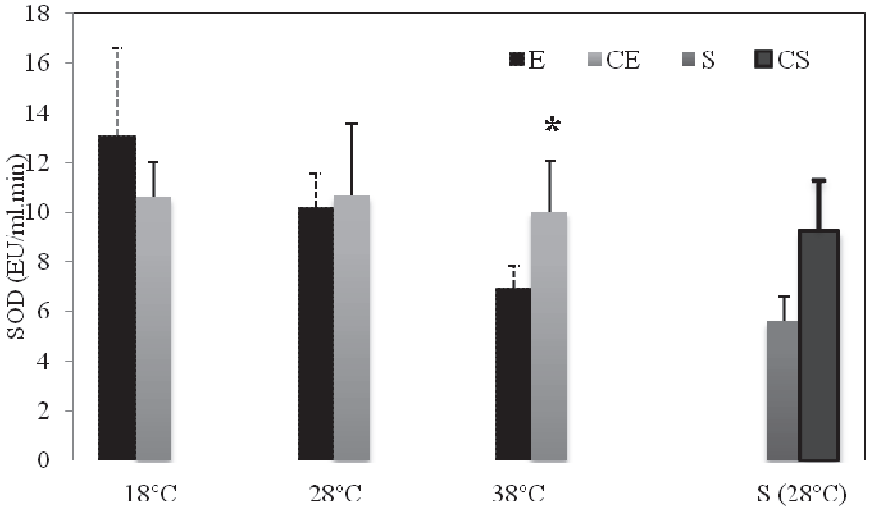

Figure 2

Figure 2. Effect of L-Carnitine on **SOD** levels in exercise and L-carnitine-exercise groups situated the same temperature. **E**, exercise groups; **CE**, L-carnitine-exercise groups; **S**, sedentary groups; **CS**, L-carnitine-sedentary groups. (**P*<0.05), CE 38°C significantly different than E 38°C.

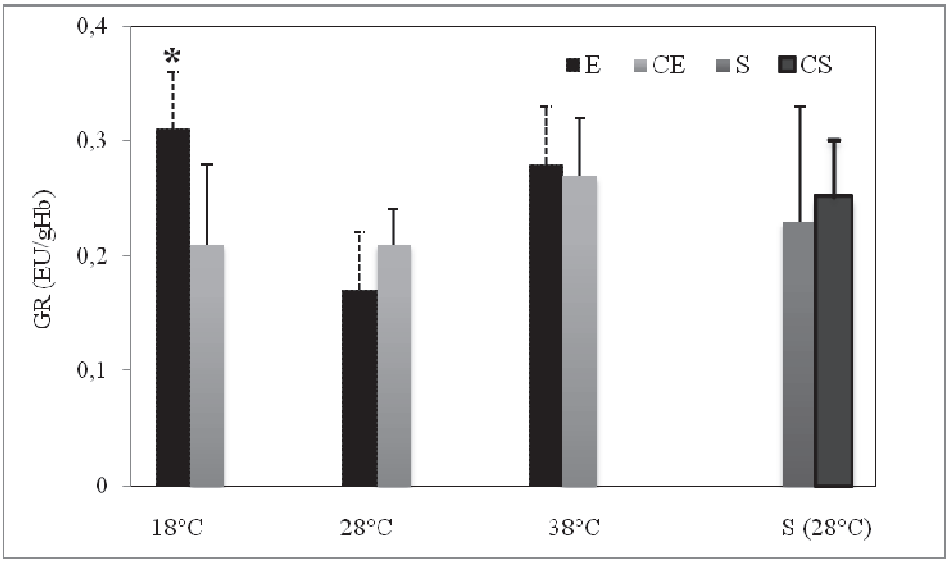

Figure 3

Figure 3: Effect of L-Carnitine on **GR** levels in exercise and L-carnitine-exercise groups situated the same temperature. **E**, exercise groups; **CE**, L-carnitine-exercise groups; **S**, sedentary groups; **CS**, L-carnitine -sedentary groups. (**P*<0.05), 18°C E significantly different than CE 18°C.

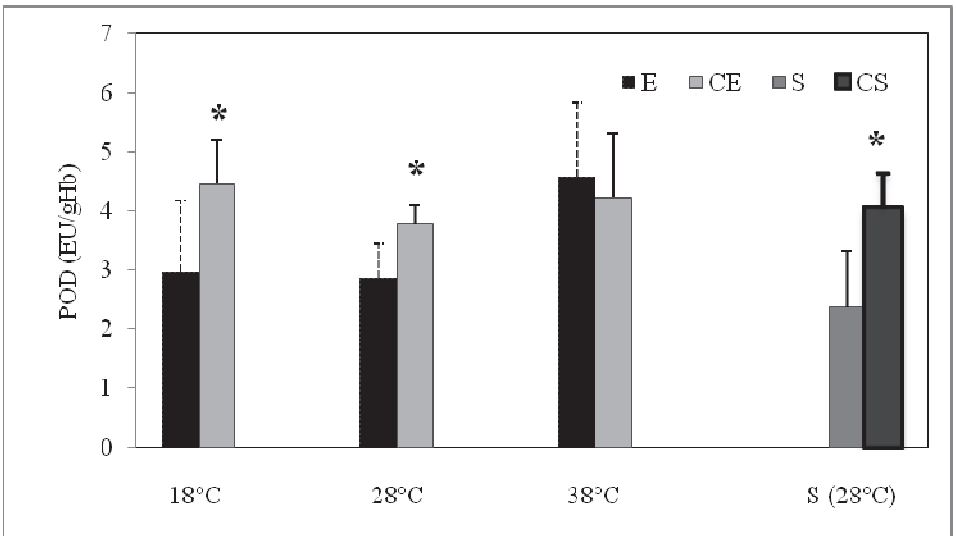

Figure 4

Figure 4: Effect of L-Carnitine on **POD** levels in exercise and L-carnitine-exercise groups situated the same temperature. **E**, exercise groups; **CE**, L-carnitine-exercise groups; **S**, sedentary groups; **CS**, L-carnitin-sedentary groups. (**P*<0.05), E 18°C significantly different than CE 18°C, E 28°C significantly different than CE 28°C, S significantly different than CS.

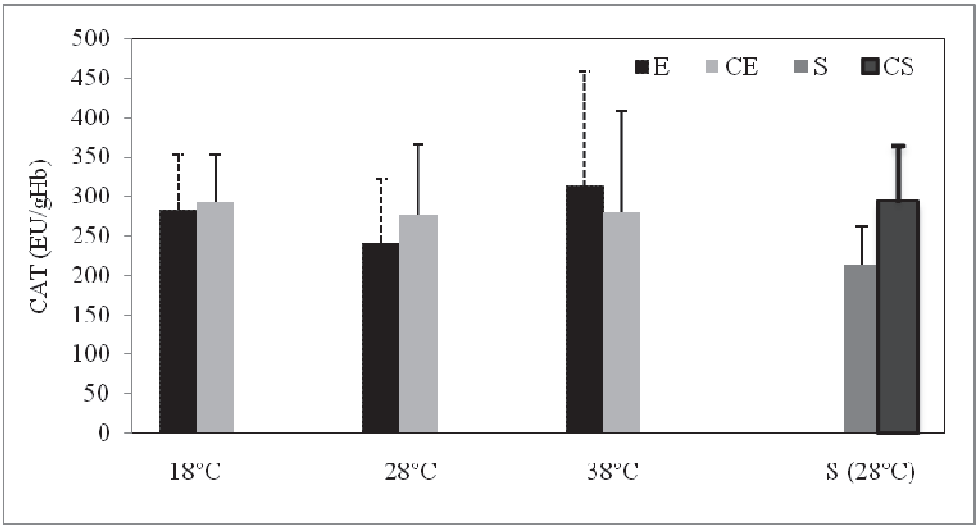

Figure 5

Figure 5: Effect of L-Carnitine on **CAT** levels in exercise and L-carnitine-exercise groups situated the same temperature. **E**, exercise groups; **CE**, L-carnitine-exercise groups; **S**, sedentary groups; **CS**, L-carnitin-sedentary groups. No significantly differences between the groups took place in the same water temperature (*P*>0.05).