Ovidius University Annals, Series Physical Education and Sport / SCIENCE, MOVEMENT AND HEALTH,
Vol. 10 ISSUE 1, 2010, Romania
Our JOURNAL is nationally acknowledged by C.N.C.S.I.S., being included in the B+ category publications, 2008-2010.
The journal is indexed in: 1. INDEX COPERNICUS JOURNAL MASTER LIST. 2. DOAJ DIRECTORY OF OPEN ACCES JOURNALS, 2009

EFFECT OF MELATONIN AND ENVIRONMENTAL STRESS ON FREE RADICALS AND ANTIOXIDANT LEVELS IN RATS DURİNG THE EXERCISE AT DIFFERENT ROOM TEMPERATURES.

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Abstract

Melatonin has a lipophilic property and is produced in a variety of organs including the pineal gland. It possesses several physiological properties that may play an important role in physiology and pathophysiology. Eight groups (E 10°C, E 23°C and E 37°C chronic treadmill exercise groups; ME 10°C, ME 23°C and ME 37°C melatonin chronic treadmill exercise groups; S 23°C and MS 23°C sedentary and melatonin-sedentary groups respectively) were formed and a total of 48 Sprague Dawley male rats, weighing 250-300 g were used in this study. In the study, the groups which took melatonin were given the related substance 5 mg/kg per day before the exercise for 4 weeks by intraperitoneally (I.P.) way. At the end of the training period (4 week) the rats were killed. Blood samples were collected for antioxidant and lipid peroxidation levels determinations. In the statistical analyses Anova, Duncan and *t*- test were used. MDA, GR levels are higher than those of ME23°C groups. GR level is higher in E10°C group compared to ME 10°C group. Serum MDA levels of the E 37°C group are lower than those of ME37°C group. Serum SOD levels are the same in E 23°C and E 37°C groups, SOD levels of both of then are lower than those of the E 10°C group. SOD levels in the sedentary and the other melatonin-sedentary group were significantly different. It was found out in the study that oxidative stress appeared most in cold environment, melatonin did not prevent oxidative stress in cold and hot environment but it activated antioxidant system and decreased emergence of free radicals at normal temperatures.

Keywords: Melatonin, rat, environmental stress, antioxidant enzymes, lipid peroxidation.

1. Introduction

Melatonin (N-acetyl-5-methoxytryptamine), the main hormone of the pineal gland, has ubiquitous actions as a direct as well as an indirect antioxidant, free radical scavenger and metal chelating effect (I.Gulçın, M.E.Buyukokuroglu, M.Oktay, 2002, Das A, A.Belagodu, R.J.Reıter, S.K.Ray, N.L.Banık, 2008), also, other organs and tissues including retina, gut, ovary, testes, bone marrow and lens have been reported to produce melatonin as well (D.X.Tan, L.C.Manchester, R.J.Reıter et al., 1999). Once formed melatonin is not stored within the pineal gland but diffuses out into the capillary blood and cerebrospinal fluid (J.Zhou, S.Zhang, X.Zhao, T.Weı, 2008). Besides directly detoxifying a variety of highly reactive molecules, melatonin also stimulates antioxidant enzymes (R.Hardeland, C.Backhaus, A.Fadavı, 2007).

Melatonin is known to influence a variety of biologic processes including the control of seasonal reproduction, circadian rhythms, influences the immune system, neuroendocrine, cardiovascular, and immune functions as well as thermoregulation (Gulçın I, Buyukokuroğlu ME, Kufrevıoğlu OI., 2003, Beydemır Ş, Gulçın I., 2004, Gulçın I., 2008). Melatonin and its metabolites show potent antioxidant ability both in vitro and in vivo, and they scavenge hydrophilic and hydrophobic reactive oxygen species (ROS) (D.X.Tan, L.C.Manchester, M.P.Terron et al., 2007). Additionally, this molecule functions in protecting cell components such as nuclear DNA, membrane lipids, and cytosolic proteins from free radical damage (R.J.Reıter, L.Tang, J.J.Garcıa, et al., 1997, S.J.Kım, R.J.Reıter, W.Qı et al., 2000).

Physical exercise is well known to be associated with cellular activation of the blood cells (F.C.Mooren, K.Volker, 2001). It was reported that by helping the regulation of energy metabolism, melatonin reduces the use of liver glycogen after exercise by increasing the liver glycogen stores in rats where exercise is done (C.D.N.B. Silva, T. Julie, Alonso-Vale Mic, et al., 2007). The influence of exercise on melatonin secretion is still a subject of intense research. In many researches melatonin secretion was explained in response to exercise since noradrenalin is necessary for at the control of increasing catecholamine secretion and melatonin synthesis during the physical exercise (A. Altun, M. Yaprak, M. Aktoz, et al., 2002, G.Gerra, I.R.Caccavar, N.Reali, et al.,1993).

A resent research in human has indicated that melatonin levels increase because of stress after exercise in long distance runners (Lopez BD, Urquijo C., 2007). Another research showed that there was no significant change in melatonin levels in the blood samples collected before exercise, during and after exercise (A.K.Baltaci, B.Cumraligil, M.Kilic et al.,2007).

Injuries in cold and hot environment (heat stroke, exhaustion, frostbite) are state that occurs during exercise. Exercise sustained at a sufficient intensity and duration may elevate core body

temperature to the upper limit of its thermoregulatory zone (T. Reilly, B. Drust, W.Gregson, 2006). Strategies which tend to reduce the body temperature before the exercise provides an important limit without reaching the body temperature vitally and make performance improve. Such improvements are shown with the reduction in body temperature by using pre-cooling maneuver before exercise (F.E.Marino, 2002).

In view of these observations, the aim of this study was to investigate the effects of melatonin on the antioxidant and lipid peroxidation levels in rats which exposed to environmental stress, such as exercise training at cold and hot conditions.

2. Material and methods

2.1. Chemicals

Melatonin, Nitroblue tetrazolium (NBT), xanthine, xanthine oxidase, oxidized glutathione, thiobarbituric acid (TBA), NADH and ABTS were obtained from Sigma company(Sigma-Aldrich GmbH, Sternheim, Germany). Ammonium thiocyanate was purchased from Merck. Trichloroacetic acid (TCA) and the other chemicals used were of 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. The rats were divided into eight equal groups. The groups which took melatonin were given the related substance 5 mg/kg per day before the exercise for 4 weeks as intraperitoneally. Body weights were measured weekly.

E 10 (Exercise, 10°C): The chronic treadmill exercise group in a laboratory at 10°C.

E 23 (Exercise, 23°C): The chronic treadmill exercise group in a laboratory at 23°C.

E 37 (Exercise, 37°C): The chronic treadmill exercise group in a laboratory at 37°C.

ME (Melatonin - Exercise, 10°C): The chronic treadmill exercise group with melatonin at pharmacological dosage (5 mg/kg) in a laboratory at 10°C.

ME (melatonin-exercise, 23°C): The chronic treadmill exercise group with melatonin at pharmacological dosage (5 mg/kg) in a laboratory at 23°C.

ME (melatonin-exercise, 37°C): The chronic treadmill exercise group with melatonin at pharmacological dosage (5 mg/kg) in a laboratory at 37°C.

S (sedentary, 23°C): The sedentary group on which no application was employed at 23±2°C (the control group).

MS (melatonin-sedentary, 23°C): This sedentary group used only melatonin and had not exercise at 23±2°C.

The groups which took melatonin were given the related substance 5 mg/kg per day before the exercise for 4 weeks intraperitoneally.

Exercise Protocol

The same exercise programs were applied to exercise and melatonin exercise groups in test groups. During the test, digital thermometer (GEMO, micro software and PID thermo controlled device) was used.

Adaptation Training: For the rats to have adaptation they were made to have exercise on treadmill for 10-15 min, at 20 m/min during per week (MAY TME 9805 treadmill exerciser, Commat Ltd., Ankara, Turkey). Adaptation training was made at 23°C laboratory temperature.

Loading Training: The exercise groups (n:36) were made to endurance training in laboratories at 10ºC, 23ºC and 37ºC. The exercise was done 6 days a week during 4 weeks. Endurance training was gradually increased training of speed and time during four weeks. A standard mild electric shock deterrent was used intermittently when necessary to coerce the rats to run. The physical training program was performed for 4 week as described in Table 1. At the end of the fourth week, rats were killed by decapitation on each training day, laboratory temperatures were regulated in accordance with exercise group temperatures (10, 23 and 37°C).

Determination of temperatures

American Health Assembly (AHA) approved normal body temperature as 36.5-37.2°C. A naked person can keep body inner temperature fixed between 12.5°C and 55°C in dry weather (SIKTAR E., 2009). For the body to feel the heat depends on the temperature of the weather, moisture rate and wind rate (FOX BOWERS FOSS., 1988). During exercise body metabolism increases 20-25 times. Unless heat loss mechanisms (radiation, conduction, convection and evaporation) function, body inner heat increases 1-1.8°C every 5 minutes and 75-90 Kcal of temperature is produced [20]. In this study the temperatures were regulated in accordance with the room temperatures at which humans and rats live (22-23°C). Moisture rate was taken into consideration and 10°C was accepted hypothermic, 37°C hiperthermic. We determined in pre-studies that the rats had difficulty under and over these temperatures. The rats tolerated up to +4°C.

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 hemolysed 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, GULÇIN I, et al., 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 (DURAK I, ANBOLAT O, KACMAZ M, et al., 1998). In this method, xanthine-xanthine oxidase complex produces superoxide radicals that react with nitroblue tetrazolium (NBT) to form a formazane (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 (DURAK I, CANBOLAT O, KACMAZ M, et al., 1998).

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 $\mu M^{-1}cm^{-1}$ (ARASHISAR Ş, HISAR O, BEYDEMIR Ş, GÜLÇIN İ. et al., 2004).

Peroxidase activity

Peroxidase activity was measured by the method of Shannon et al. (SHANNON LM, KAY E., LAW JY. 1996). For this purpose, 0.1 mL hemolysate was taken up into potassium phosphate buffer (1.7 mL, 0.05 M, pH 7.3). Then 0.5 mL ABTS was added. The reaction was started by addition of H_2O_2 (0.2 mL, 0.2M). Change in absorbance at 470 nm was recorded for 2 min at intervals of 15 sec. The enzyme activity was expressed as enzyme unit.$min^{-1}gHb^{-1}$ (ARASHISAR Ş, HISAR O, BEYDEMIR Ş, GÜLÇIN İ. et al., 2004).

Glutathione Reductase Activity

Glutathione reductase activity was estimated by the method of (GOLDBERG D.M, SPOONER RJ., 1983). To 0.1 mL hemolysate 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 oxidized 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 oxidized $min^{-1}.gHb^{-1}$ protein using the molar extinction coefficient 6.23 $\mu M^{-1}cm^{-1}$. (ARASHISAR Ş, HISAR O, BEYDEMIR Ş, GÜLÇIN İ. et al., 2004).

Determination of MDA

MDA concentrations in blood plasma samples were measured by the high-performance liquid chromatography with fluorescent detection (HPLC-FLD). Briefly 50 µL 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 alkaline methanol was added to the sample, vortex, centrifuged (3000xg for 3 min), and the aqueous layer was removed.

Then, 20 µL supernatant was analyzed by HPLC (HP, Agilent 1100 modular 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) standard solution preceded in exactly the same as plasma and urine sample (KHOSCHSORUR GA, WINKLHOFER-ROOB BM, RABL H, et al., 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

Melatonin caused a significant increase in the MDA levels of the E 23°C group compared with the ME 23°C group *(P*<0.05), and the ME 37°C group, compared with the E 37°C group *(P*<0.05) (The mean of standard derivations of groups respectively, 0.45±0.08 and 0.26±0.13; 0.29±0.06 and 0.45±0.09). There was no difference between the E 10°C group and the ME 10°C group and between the S 23°C group and the MS 23°C group in Fig.1.

The SOD values for serum increased significantly in the ME 23°C group according to the E 23°C group (P<0.05) and the MS 23°C group according to the S 23°C group (P< 0.001). The mean and standard deviation of ME 23°C and E 23°C groups was determined respectively as 6.56±0.778 and 8.26±1.06, and E 23°C and MS 23°C groups as 5.61 ± 1.04 and 9.34 ± 0.65. The SOD values were not different significantly between the others groups in Fig.2.

In Fig. 3, GR levels decreased significantly the ME 10°C than the E 10ºC group (P<0.05) and the ME 23°C group than the E 23°C group (P<0.05). While values of ME 10ºC and E 23°C groups were found as 0.39 ± 0.08 and 0.27±0.07, the values of ME 23°C and E 23ºC groups were determined as 0.33±0.04 and 0.25±0.05. Not significant between E 37°C and ME 37°C and S 23°C and MS 23°C. POT and CAT values were not significant in any groups in the same temperature (Fig. 4 and 5).

According to the effect of heat stress, MDA levels of E 10°C group were significantly different from E 23°C group and E 37°C group. E 23°C group was significantly different from E 37°C group. Also, SOD levels increased a significant E 10°C group compared with E 23°C group and E 37°C group. MDA levels, ME 10°C group increased significantly according to ME 23°C group and ME 37°C group, and ME 23°C group decreased from ME 37°C group. GR, POD and CAT levels is not significant both exercise groups and melatonin-exercise groups in different temperature in Tab.2.

4. Discussion

The aim of this study was to investigate the effects of environmental stress like prolonged training on antioxidant and lipid peroxidation levels. According to the effect of melatonin, the highest MDA levels were obtained in 23°C exercise group and 37°C melatonin-exercise group in the present study (Fig.1). SOD level increased in 23°C melatonin-exercise group, and GR level increased significantly in 23°C and 10ºC exercise groups (Fig 2 and 3).

During the normal aerobic, mechanism form reactive oxygen species (ROS) such as superoxide anion and hydroxyl radical in the body (Y.J. Kim, J.E. Chung, M.Kurisawa, H.Uyama, et al., 2004). Physical activity increases the generation of reactive oxygen species in several ways. As oxidative phosphorylation increases in response to exercise, Catecholamines that are released during exercise, including prostanoid metabolism, xanthine oxidase, NAD(P)H oxidase, and several secondary sources, such as the release of radicals by macrophages recruited to repair damaged tissue, there will be a concomitant increase in free radicals (J.Peake, K.Suzuki, 2004, M.L.Urso, P.M.Clarkson. 2003). During exercise, tissue injury may result from muscle damage, thermal stress and ischaemia/reperfusion. When produced in excess, neutrophil-derived ROS may overwhelm the body's endogenous antioxidant defense mechanisms, and this can lead to oxidative stress observed that aerobic training decreased lipid peroxidation (levels of TBARS) under high intensity exercise regimes (J.Peake, K.Suzuki, 2004, F.Marzatico, O.Pansarasa, L.Bertorelli et al. 1997, M.M.Kanter , L.A.Nolte, J.O.Holloszy, 1993).

This study has indicated that SOD, increased particularly in cold environment, induced more oxidative stress according to the other temperatures (Price MJ, Campbell I.G. 2002), pointed out that continuous increases in aural temperature, skin temperature and heat storage were observed during exercise in warm conditions when compared with cool conditions, indicating an increased thermal strain during exercise, although the thermal stress remained constant.

The reason that not among melatonin groups is a significant may proceed from antioxidant effect of melatonin. Melatonin, released from the pineal gland had role for regulation of several biological functions such as sleep, reproduction, circadian rhythm and immune function (A.Brzezinski, 1997), cells and tissues protect against oxidative damage (A.Brzezinski , 1997, R.J.Reiter, 1993). Additionally, melatonin affects renal function, acting directly on water and electrolyte function (E.Skotnicka, A.J.Hynczak, 2001) preserve glycogen stores in exercised rats through the mediation of carbohydrate and lipid utilization (R.C.Mazepa, M.J.Cuevas, P.S.Collado, et al., 1999), and increase the exercise-induced secretion of growth hormone in humans (D.R.Meeking, J.D.Wallace, R.C.CUNEO, et al., 1999). There have been some researches concerning the *in vitro* and *in vivo* antioxidant properties of melatonin (M.Koç, S.Taysi, M.E. Buyukokuroğlu, N. Bakan. 2003, S.TAYSI, H.Ucuncu, M.Elmastas, et al. 2005, R.J.Reiter, L.Tang, J.J.Garcia et al**.** 1997), It was observed that the function of melatonin removing hydroxyl radical stronger 5 times than glutathione and 15 times than mannitol, and that the function of its scavenger peroxide radical stronger 2 times more than vitamin E (R.J.Reiter, 1996). However, its antioxidant and anti-inflammatory effect have not been searched in exercise performance.

The ergogenic effect of melatonin is limited on exercise performance in literature. Mclellan et al. (TM Mclellan, IF Smith, GA Gannon, et al., 2000), investigated the effect of 2x1 mg doses of melatonin on the thermo-regulatory responses and tolerance time to intermittent treadmill walking at 3.5 km/h in a environment chamber at 40ºC, and concluded that this low dose of melatonin had no impact on tolerance to uncompensable heat stress and that trials conducted in the early afternoon were associated with an increased rectal temperature tolerated at exhaustion that offset the circadian influence on resting rectal temperature and thus maintained tolerance times similar to those of trials conducted in the morning. In another study, McClellan et al. (R.A.L. Osorio, J.S. ChristofanI, VD Almeida et al., 2003) examined the effect of 5 mg dose

of melatonin induced a lower rectal temperature response at rest in both a cool (at 23°C) and hot environment (at 40°C).They reported that the small decrease in rectal temperature following the ingestion of 5 mg of melatonin at rest in a cool environment had no influence on subsequent tolerance during uncompensable heat stress.

In results of this study, while it has useful effect of melatonin in normal temperature, increasing in MDA levels has been thought to have a negative effect in hot environment. A research suggest that hyperthermia in sedentary rats presents smaller effect over lipid peroxidation than hypothermia (Osorio RAL, ChristofanI JS, Almeida VD et al., 2003).

According to the effect of heat stress, both at 10°C exercise groups and at 10°C melatonin-exercise groups were attained in the highest of MDA levels (Tab. 1). These results indicated that in a cold environment occurred more stress than the other environment temperatures (at 23°C and 37°C). Exogenous melatonin administration has a clear hypothermic effect under resting conditions (A. CagnaccI, R. Soldani, C. Romoglono., 1994, C.V. Van Den Heuvel, D.C. Kennaway, D.Dawson, 1999). While (A.CagnaccI, J.A.Elliott, S.S.C.Yen, 1992), Cagnacci and co-workers determined that 2.5 mg dose of melatonin was reduced 0.3°C body temperature at rest on female, the other researches reported that 0.1, 0.3, 1 and 10 mg doses of melatonin were decreased the body temperature 0.12°C and 0.2°C respectively in individuals who were given 1 and 10 mg doses of melatonin (S.Aizawa, H.Tokura, T.Morita, 2002) and (Reid K, C.Van Den Heuvel, D.Dawson, 1996) examined the change of body temperature was 0.28°C (5 mg dose of melatonin). Also, it was conceived that cold exposures in dark significantly decreased melatonin levels in pineal glands and serum in quails (P.P.Lee, A.E.Allen, S.F.Pang, 1990).

There were some adverse researches reporting that melatonin increased in literature (T.M.Lee, W.G.Holmes, I.Zucker, 1990). Likewise, conclude that pharmacological doses of melatonin induce hypothermia in hens by increasing nonevaporative skin heat losses and slightly increasing respiratory evaporation in chicken (I.Rozenboim, L.Miara, D.Wolfenson, 1998).

With respect to this knowledge, it may be thought melatonin does not prevent the oxidative damage relating to that it fall off body temperature and decrease its quantity in cold environment. However, it has not known completely hypothermic effect of melatonin on exercise. Atkinson et al. declared that the administration of exogenous melatonin leads to hypnotic and hypothermic responses in humans, which can be linked to immediate reductions in short-term mental and physical performance (G.Atkinson, B.Drust, T.Reilly et al., 2003). These effects may still be apparent 3-5 hours after administration for some types of cognitive performance, but effects on physical performance seem more short-lived depending on the dose of melatonin. Furthermore, they stressed that the hypothermic effects of melatonin lead to improved endurance performance in hot environments is not supported by evidence from studies involving military recruits who exercised at relatively low intensities.

As a conclusion, it was found out in the study that oxidative stress appeared most in cold environment, melatonin did not prevent oxidative stress in cold and hot environment but it activated the antioxidant system and decreased emergence of free radicals at normal temperatures.

REFERENCES

ALTUN, A, YAPRAK, M, AKTOZ, M, et al. 2002, *Impaired nocturnal synthesis of melatonin in patients with cardiac syndrome* X. Neurosci Lett 2002; 327: 143–145.

AEBI, H. 1984, Catalase in vitro. Methods Enzymol 1984; 105: 121-126.

ARASHISAR, Ş., HISAR, O., BEYDEMIR, Ş., GÜLÇIN İ. et al., **2004**, Effect of vitamin E on carbonic anhydrase enzyme activity in rainbow trout (Oncorhynchus mykiss) erythrocytes in vitro and in vivo. Acta Vet Hung; 52: 413-422.

AIZAWA, S., TOKURA, H., MORITA, T., 2002, *The administration of exogenous melatonin during the daytime lowers the thermoregulatonousry setpoint.* J Therm Biol; **27:** 115-119.

ATKINSON, G., DRUST, B., REILLY, T. et al., 2003, *The relevance of melatonin to sports medicine and science.* Sports Med; 33: 809-831.

BEYDEMIR, Ş., GULÇIN, I., 2004, *Effect of melatonin on carbonic anhydrase from human erythrocyte in vitro and from rat erythrocyte in vivo.* J Enzym Inhib Med Chem; 19: 193–197.

BALTACI, AK., CUMRALIGIL, B., KILIC, M., et al. 2007, *Effect of acute swimming exercise on lactate levels and its relation with zinc in pinealectomized rats.* Cell Biochem Funct 25: 597–601.

BRZEZINSKI, A., 1997, *Melatonin in humans.* N Engl J Med 1997; 336: 186-95.

CAGNACCI, A., ELLIOTT, JA., YEN, SSC., 1992, *Melatonin: a major regulator of the circadian rhythm of core temperature in humans.* J Clin Endocrinol Metab 1992; 75**:** 447-452.

CAGNACCI, A., SOLDAN,I R., ROMOGLONO, C., 1994, *Melatonin induced decrease of body temperature in women: a threshold event*. Neuroendocrinology 1994; 60: 549-52.

DAS, A., BELAGOD,U A., REITE, RJ. RAY, SK., BANIK, NL., 2008, *Cytoprotective effects of melatonin on C6 astroglial cells exposed*

to glutamate excitotoxicity and oxidative stress. J Pineal Res 2008; 45:117–124.

DURAK, I., CANBOLAT, O., KACMAZ, M., et al.1998, *Antioxidant interferences in superoxide dismutase activity methods using superoxide radical as substrate. Clin Chem* Lab Med 1998; 36: 407-408.

FOX, BOWERS. FOSS., 1998, *The physiological basis of physical education and athletics.* W.B. Saunders Company Fourth Edition.1988.

GERRA, G., CACCAVARI., REALI, N., et al. 1993, *Noradrenergic and hormonal responses to physical exercise in adolescents. Relationship to anxiety and tolerance to frustration.* Neuropsychobiology 1993; 27: 65-71.

GOLDBER, D.M, SPOONER, R.J., 1983, *Glutathione reductase*. In: Bergmayer, O.H. (ed.) Methods of Enzymatic Analysis. pp. 1983; 259-65.

GULÇIN, I., BUYUKOKUROGL, U. M., OKTAY, M., et al, **2002**, *On the in vitro antioxidant properties of melatonin.* J Pineal Res 2002; 33: 167-171.

GULÇIN, I., BUYUKOKUROĞLU, M.E., KUFREVIOĞL,U O.I., **2003,** *Metal chelating and hydrogen peroxide scavenging effects of melatonin.* J Pineal Res 2003; 34: 278-281.

GULÇIN, I., 2008, *Measurement of antioxidant ability of melatonin and serotonin by the DMPD and CUPRAC methods as trolox equivalent*. J Enzym Inhib Med Chem, 2008; 23, 871–876.

HARDELAND, R., BACKHAU, C., FADAVI, A., 2007, *Reactions of the NO redox forms NO^+, NO and HNO (protonated NO^-) with the melatonin metabolite N-acetyl-5-methoxykynuramine.* J Pineal Res 2007; 43: 382–388.

KANTER, M.M., NOLTE, L.A, HOLLOSZ, J.O., 1993, *Effects of an antioxidant vitamin mixture on lipid peroxidation at rest and post exercise.* J Appl Physiol, 74: 965-969.

KHOSCHSORUR, G.A, WINKLHOFER-ROOB B.M., **RABL, H., et al**., **2000**, *Evaluation of a sensitive HPLC method for the determination of malondialdehyde, and application of the method to different biological materials.* Chromatographia, 52: 181-4.

KIM, Y.J., CHUNG, J.E, KURISAWA, M., UYAMA, H., et al., **2004**, *Superoxide anion scavenging and xanthine oxidase inhibition of (+)-catechin-aldehyde polycondensates. Amplification of the antioxidant property of (+)-catechin by polycondensation with aldehydes.* Biomacromolecules, 5: 547-552.

KI, S..J, REITER, R.J,. QI, W., et al., **2000,** *Melatonin prevents oxidative damage to protein and lipid induced by ascorbate-Fe^{3+}-EDTA: Comparison with glutathione and a-tocopherol.* Neuroendocrinol Lett, 21: 269–276.

KOÇ, M., TAYSI, S., BUYUKOKUROĞLU, M.E., BAKAN, N., 2003*, Melatonin protects rat liver against irradiation- induced oxidative injury.* J Radiat Res, 44: 211-215.

LOPEZ, B.D., URQUIJO, C., 2007, *Influence of physical training on the Syrian hamster's melatonin rhythm.* Neurosci Lett, 428: 68–71.

LEE, P.P., ALLEN, A.E., PANG, S.F, 1990, *Cold stress during scotophase elicited differential responses in quail pineal, retinal, and serum melatonin levels.* Acta Endocrinol, 122: 535–539.

LEE, T.M, HOLMES, W.G, ZUCKER, I., 1990, *Temperature dependence of circadian rhythms in golden-manted ground squirrels*. J Biol Rhythms, 5**:** 25-34.

MOOREN, F.C., VOLKER, K., 2001, *Exercise-induced modulation of intracellular signaling pathways.* Exerc Immunol Rev 7: 32–65.

MARINO, F.E., 2002, *Methods, advantages and limitations of body cooling for exercise performance.* Br J Sports Med, 36: 89-94.

MARZATICO, F., PANSARASA, O., BERTORELLI, L., et al., 1997, *Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes.* J Sports Med Phys Fitness, 37: 235-239.

MAZEPA, R.C., CUEVA, M.J., COLLADO, P.S, et al., 1999, *Melatonin increases muscle and liver glycogen content in nonexercised and exercised rats.* Life Sci, 66: 153-60.

MEEKING, D. R., WALLACE, J.D, CUNEO RC, et al. 1999, *Exercise-induced GH secretion is enhanced by oral ingestion of melatonin.* Eur J Endocrinol, 141: 22-26.

MCLELLAN, T.M., GANNON, G.A., ZAMECNIK, J. et al., **1999**, *Low doses of melatonin and diurnal effects on thermoregulation and toler-alleviance to uncompensable heat stress*. J Appl Physiol 1999; 87: 308-316.

MCLELLAN, T.M, SMITH, I.F, GANNON, G.A, et al. *2000,* *Melatonin has no effect on tolerance to uncompensable heat stress in man.* Eur J Appl Physiol 2000; 83: 336-43.

OLCAY, H., BEYDEMIR, Ş., GULÇIN, I., et al. 2005, *Effects of low molecular weight plasma inhibitors of rainbow trout (Oncorhynchus mykiss) on human erythrocyte carbonic anhydrase isozyme*

activity in vitro and rat erythrocytes in vivo. J. Enzym Inhib Med Chem 2005; 20: 35-39.

OSORIO, RAL., CHRISTOFANI, J.S., ALMEIDA, V.D. et al., 2003, *Reactive oxygen species in pregnant rats: effects of exercise and thermal stress* Comparative Biochemistry and Physiology Part C 2003; 135: 89–95.

PRICE, M.J., CAMPBELL, I.G., 2002, *Thermoregulatory responses during prolonged upper-body exercise in cool and warm conditions.* J Sports Sci 2002; 20: 519-27.

PEAKE, J., SUZUKI, K,. 2004, *Neutrophil activation, antioxidant supplements and exercise-induced oxidative stress.* Exerc Immunol Rev 2004; 10: 129-41.

REITER, R.J., TANG, L., GARCIA, J.J., et al. 1997, *Pharmacological action of melatonin in oxygen radical pathophysiology.* Life Sci 1997; 60: 2255–2271.

REILLY, T., DRUST, B., GREGSON, W., 2006, *Thermoregulation in elite athletes.* Curr Opin Clin Nutr Metab Care 2006; **9:** 666–671.

REITER, R.J., 1993, *Interactions of the pineal hormone melatonin with oxygen-centered free radicals:* A brief review. Brazal J Med Biol Res 1993; 26**:** 1141-55.

REITER, R.J., TANG, L., GARCIA, J.J., et al. 1997, *Pharmacological actions of melatonin in oxygen radical pathophysiology.* Life Sci 1997; 60: 2255-2271.

REITER, R.J., 1996, *Functional diversity of the pineal hormone melatonin: its role as an antioxidant.* Exp clin Endocrinol, 104: 10-6.

REID, K., VAN DEN HEUVEL, C., DAWSON, D., 1996, Day-time melatonin administration: effects on core temperature and sleep onset latency. J Sleep Res, 5: 150-154.

ROZENBOIM, I., MIARA, L., WOLFENSON, D., 1998, *The thermoregulatory mechanism of melatonin-induced hypothermia in chicken.* Am J Physiol Regulatory Integrative Comp Physiol, 274: 232-236.

SKOTNICKA, E., HYNCZAK, A.J., 2001, *Melatonin and its possible role in regulation of water and electrolyte metabolism*. Med Weter, 57**:** 299-303.

SILVA, C.D.N.B., JULIE, T., ALONSO-VALE, MIC. et al. 2007, *Pinealectomy reduces hepatic and muscular glycogen content and attenuates aerobic power adaptability in trained rats.* J Pineal Res 2007; 43: 96–103.

SIKTAR, E., 2009, *The effect of L-carnitine on carbonic anhydrase level in rats exposed to exhaustive exercise and hypothermic stress*. 2009 8(13): 3060-3065.

SHANNON, L.M., KAY, E., LAW, J.Y., 1996, *Peroxidase isoenzymes from horseradish roots: isolation and physical properties*. J Biol Chem 1996: 241; 2116-2172.

TAN, D.X., MANCHESTER, L.C., REITER, R.J. et al., 1999, *Identification of highly elevated levels of melatonin in bone marrow: its origin and significance.* Biochim Biophys Acta 1999; 1472: 206–214.

TAN, D.X., MANCHESTER, L.C., TERRON, M.P. et al. 2007, *One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species?* J Pineal Res, 42: 28-42.

TAYSI, S., UCUNCU, H., ELMASTAS, M., et al. 2005, *Effect of melatonin on lipid peroxidation, glutathione and glutathione-dependent enzyme activities in experimental otitis media with effusion in guinea pigs.* J Pineal Res, 39: 283-286.

URSO, M.L., CLARKSON, P.M., 2003, *Oxidative stress, exercise, and antioxidant supplementation*. Toxicology,189: 41-54.

VAN DEN HEUVEL, C.V., KENNAWAY, D.C., DAWSON D. 1999, *Thermoregulatory and soporific effects of very low dose melatonin injection.* Am J Physiol Endocrinol Metab 276: 249-254.

ZHOU, J., ZHANG, S., ZHAO, X., WEI, T., 2008, *Melatonin impairs NADPH oxidase assembly and decreases superoxide anion production in microglia exposed to amyloid-β_{1-42}*. J Pineal Res, 45: 157–165.

Exercise week	Exercise time (min)	Exercise speed (m/min)
1	30	23
2	40	23
3	50	25*
4	60	25*

Table 1: The exercise was done 6 days a week during four weeks. Training of speed and time gradually increased during four weeks. *2% uphill gradient

	Exercise Groups			Melatonin –Exercise Groups	
Parameter	Temperature (°C)	Mean ± S.D.	P	Mean ±S.D.	P
MDA (nmol/ml)	10	0.78 ± 0.13[a]	0.000*	0.81 ± 0.12[a]	0.000***
	23	0.45 ± 0.08[b]		0.26 ± 0.13[c]	
	37	0.29 ± 0.06[c]		0.45 ± 0.09[b]	
SOD (EU/ml.min)	10	10.4 ± 1.73[a]	0.000**	9.12 ± 2.56	0.549
	23	6.56 ± 0.77[b]		8.26 ± 1.06	
	37	5.33 ± 0.79[b]		7.66 ± 2.40	
GR (Eu/gHb)	10	0.39 ± 0.08	0.110	0.27 ± 0.07	0.603
	23	0.33 ± 0.04		0.25 ± 0.05	
	37	0.30 ± 0.05		0.23 ± 0.04	
POD (EU/gHb)	10	1.83 ± 0.32	0.675	2.00 ± 0.32	0.328
	23	1.83 ±0.33		1.70 ± 0.36	
	37	1.99 ± 0.34		1.86 ± 0.24	
CAT (Eu/gHb)	10	274.84±42.44	0.060	249.56±62.85	0.243
	23	225.05±13.21		198.80±48.30	
	37	239.57±34.66		217.59±29.20	

Table 2: Effect of heat stress on values of MDA, SOD, GR, POD and CAT in exercise and melatonin-exercise groups at different temperature
*P<0.001: significantly differences in among a>b>c exercise groups. **P<0.001: significantly difference beetwen a and b exercise groups. ***P<0.001: significantly difference among a>c>b melatonin-exercise groups.

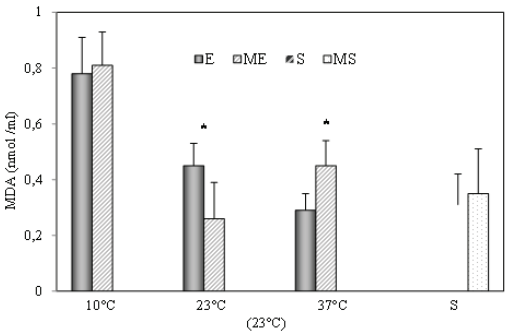

Figure 1.Effect of melatonin on MDA levels in exercise and melatonin-exercise groups situated the same temperature. (E, exercise groups; ME, melatonin-exercise groups; S, sedentary groups; MS, melatonin-sedentary groups.) (* *P*<0.05), significantly different E 23ºC than ME 23ºC and ME 37ºC than E 37ºC.

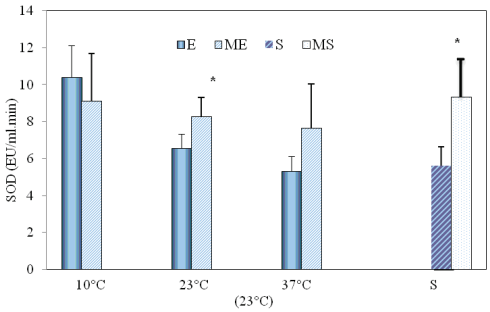

Figure 2. Effect of melatonin on SOD levels in exercise and melatonin-exercise groups situated the same temperature. (E: exercise groups, ME: melatonin-exercise groups, S: Sedentary groups, MS: Melatonin-sedentary groups.) (* *P*<0.05), significantly different ME 23ºC than E 23ºC and MS 23ºC than S 23ºC.

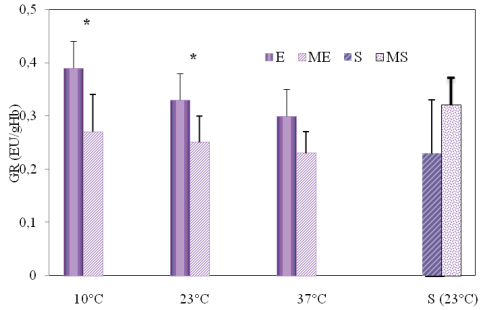

Figure 3: Effect of melatonin on GR levels in exercise and melatonin-exercise groups situated the same temperature. (E, exercise groups; ME, melatonin-exercise groups; S, sedentary groups; MS, melatonin-sedentary groups.) (*P<0.05), significantly different E 10ºC than ME 10ºC and E 23ºC than ME 23ºC.

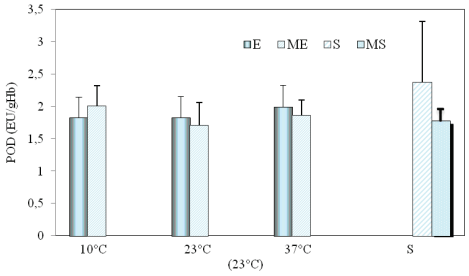

Figure 4: Effect of melatonin on POD levels in exercise and melatonin-exercise groups situated the same temperature. (E, exercise groups; ME, melatonin-exercise groups; S, sedentary groups; MS, melatonin-sedentary groups.) (*P*>0.05), No significantly difference among groups in same temperature.

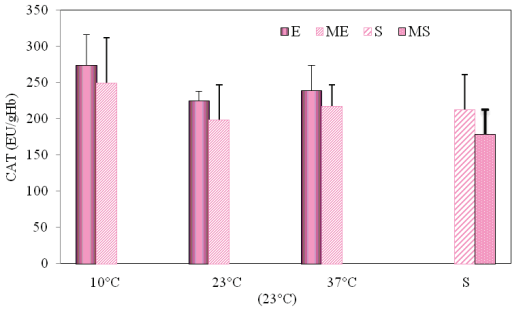

Figure 5: Effect of melatonin on CAT levels in exercise and melatonin-exercise groups situated the same temperature. (E: exercise groups; ME, melatonin-exercise groups; S, sedentary groups; MS, melatonin-sedentary groups.). (*P*>0.05), No significantly difference among groups in same temperature.