Asian Journal of Chemistry Vol. 22, No. 1 (2010), 705-713

Effect of Magnetic Field on Plasma Zn, Cu and Malondialdehyde Levels and Na^+-K^+ Atpase Activity of Isolated Diaphragmatic Muscle in a Diabetic Rat Model

A. Demirkazik*, G. Yucebilgic†, M. Emre‡, A. Pelit‡,
A. Cetin§, S. Gulturk¶ and S. Kavak#
Departments of Biophysics, Cumhuriyet University, School of Medicine, 58140 Sivas, Turkey
Fax: (90)(346)2191955; Tel: (90)(346)2191000; E-mail: dmrkzk@yahoo.com

The aim of this study is to investigate the effect of magnetic field (MF) on plasma Zn, Cu and malondialdehyde (MDA) levels and Na^+-K^+ ATPase activity of isolated diaphragmatic muscle in a diabetic rat model. Rats allocated as control (n = 10), diabetes mellitus (DM) (n = 10), MF-exposed (MFE) (n = 10) and DM plus MF-exposed (DM-MFE) (n = 10) groups. After induced DM, the MF were exposed to MF in solenoid 165 minutes every day during 1 month. Cardiac blood was obtained and the plasma Zn, Cu and MDA levels and Na^+- K^+ ATPase activity of diaphragmatic muscle were determined. Plasma Cu level of the control was significantly lower than those of the DM and MFE ($p < 0.05$). Plasma Zn level of the DM-MFE was significantly lower than those of the control, DM and MFE ($p < 0.05$). Plasma MDA level of the DM was significantly higher than those of the control, MFE and DM-MFE ($p < 0.05$). Diabetes mellitus and magnetic field increase plasma Cu level and do not affect plasma Zn level, but MF plus DM decreases its level. While DM increases plasma MDA level, MF has opposing effect on MDA level. Diabetes mellitus decreases Na-K ATPase of rat diaphragmatic muscle and MF elevates its level to normal.

Key Words: Copper, Magnetic field, Malondialdehyde, Na-K Atpase, Zinc.

INTRODUCTION

Biological effects of magnetic fields (MFs) raise the question of whether imposed magnetic fields constitute a hazard in terms of physiological processes. Besides, the development of diagnostic and therapeutic applications of magnetic field draws attention to possible effects. There are several reports of positive clinical effects

†Department of Chemistry, Cukurova University, School of Science and Literature, 01140 Adana, Turkey.

‡Departments of Biophysics, Cukurova University, School of Medicine, 01140 Adana, Turkey.

§Department of Obstetrics and Gynecology Physiology, Cumhuriyet University School of Medicine, 58140 Sivas, Turkey.

¶Department of Physiology, Cumhuriyet University School of Medicine, 58140 Sivas, Turkey.

#Departments of [3]Biophysics and [6]Medical Biology, Cukurova University School of Medicine, 01140 Adana, Turkey.

after exposure to applied magnetic fields. Liboff and Jenrow[1] reported that there were physical mechanisms in neuroelectromagnetic therapies. Most functions in the human body are tightly controlled and activation of the processes underlying these functions must take place at the cellular level. Nervous and hormonal systems are two important communication systems in the body affecting cellular functions. Electromagnetic field (EMF) induces ion migration in biological tissues, changes in their concentration in various cell compartments and intracellular space and polarization of biomolecules and breakage of hydrogen bonds. Many researchers have shown that electromagnetic fields increase the average concentration of free radicals, lengthen their lifetime and enhance the probability of radical reactions with cellular components[2-4]. It has been reported that exposure to magnetic field induced changes in the activity of some enzymes involved in the antioxidant system and thiol-disulfide exchange in the liver of animals[5]. Zinc is a trace element, essential for living organisms. More than 300 enzymes require zinc for their activity. Furthermore, zinc deficiency increased the lipid peroxidation in various rat tissues, whereas the zinc supplementation corrected the impairment[6,7].

Increased oxidative stress as measured by indices of lipid peroxidation and protein oxidation has been shown to be increased in both insulin dependent (IDDM) and non-insulin dependent (NIDDM) diabetes mellitus (DM)[8] and it could cause initial β cell damage in type I DM or impaired insulin production, release or function in type II diabetes mellitus[9-11]. Several complications of diabetes mellitus may be related to increased intracellular oxidant and free radicals associated with decreases in intracellular zinc and zinc dependent antioxidant enzymes because zinc is widely described as an antioxidant[12-14]. Zinc-deficient animals have reduced glucose tolerance, lowered serum insulin content and elevated total insulin-like activity after glucose stimulation, when compared to pair-fed controls[13,15,16]. Also, Cu-Zn superoxide dismutase (SOD) is an important enzyme related to scavenging of reactive oxygen species. Although copper is crucial for catalytic activity and zinc has structural role, the latter metal is necessary for the full activity of the enzyme. Cu-Zn SOD has been reported to be lower in zinc deficiency[16]. In the literature, there was no study investigating the effect of MF on plasma Zn, Cu and MDA levels which are related to lipid peroxidation and on Na^+-K^+ ATPase activity of isolated diaphragmatic muscle for evaluation of skeletal muscle function in diabetic subjects exposed to magnetic field.

The aim of this study is to investigate the effect of magnetic field on plasma Zn, Cu and MDA levels and Na^+-K^+ ATPase activity of isolated diaphragmatic muscle in a diabetic rat model.

EXPERIMENTAL

Tissue preparation: All procedures were approved and performed under guidelines off the Animal Ethics Committee of Cukurova University School of Medicine. A total of 40 male Wistar rats, weighing between 220-260 g, were used in this study. The diaphragmatic muscle of these rats, killed by decapitation, was prepared according to the method described by Kelsen and Nochomovitz[17].

Experimental protocols: Four sets of experimental studies were performed and the rats allocated randomly into 4 groups: control (n = 10), diabetes mellitus (DM) (n = 10), MF-exposed (MFE) (n = 10) and DM plus MF-exposed (DM-MFE) (n = 10) groups. They were kept in individual stainless steel cages in air conditioned rooms maintained at 21 °C ± 4 with a 12 h light-12 h dark cycle. Food and water were provided *ad libitum*.

Induction of diabetes: Diabetes mellitus was induced by a single tail vein injection of streptozotocin (45 mg/kg body weight; Sigma, St. Louis, MO. USA) under halothane anesthesia. Diabetes mellitus was confirmed 24-48 h later by the presence of hyperglycemia detected in a drop of urine by using glucotest sticks (184047 Roche Diagnostics Scandinavia AB, Basel, Switzerland). Rats were randomly divided into four groups: control unexposed (C, n=10), control exposed magnetic field (CMF, n = 10), diabetic (D, n = 10) and diabetic exposed magnetic field (DMF, n = 10).

Application of modulation magnetic field: The magnetic field was generated in a specially designed device which developed in the Department of Biophysics, University of Cukurova in Adana in Turkey. The device had a solenoid of 500 mm in length and 210 mm in diameter. This magnet was constructed by winding 1400 turns of an insulated soft copper wire, which was 1.4 mm in diameter on a fiber base. The alternating current passing through the wires was frequency of 50 Hz electricity current (50 Hz) was passed through the device and a time relay was added into the system. In this way, the alternating magnetic field was exposed on the rats for 30 min and it was halted for 15 min within every 165 min. The magnetic field intensity was measured as B = 5 mT every point the solenoid. Due to fact that solenoid poles were winded more than 1400 turns since the intensity in the poles are usually weaker than it is in the center of the solenoid. Magnetic field intensity was measured by a digital teslameter (PHYWE Systeme GmbH, Göttingen, Germany) with an axial Hall-effect probe. The solenoid was always kept in a north-south direction and its temperature was maintained constant at 25 °C (Fig. 1). The exposure was applied for four weeks for MF and DM-MFE groups.

Inside the solenoid and between the poles of the electromagnet, a cage (40 cm × 17 cm × 13 cm) made of Plexiglas was placed in the homogeneous magnetic field. The cage was perforated to permit air passage for breathing was used. Five experimental animals were placed, in order to be MF exposed, in this cage and exposure was always applied in a separate department apart from that for the control group and the animals were not exposed to electrical transients when the field was turned on and off.

Five mL blood was collected from intracardiac non-fasting rats final experiment before the rats were sacrificed by decapitation[17]. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) an end product of fatty acid peroxidation. The level of MDA was determined by measuring the colour intensity of the complex formed between MDA and thiobarbituric acid at 532 nm according the method of Ohkawa and Yagi[18].

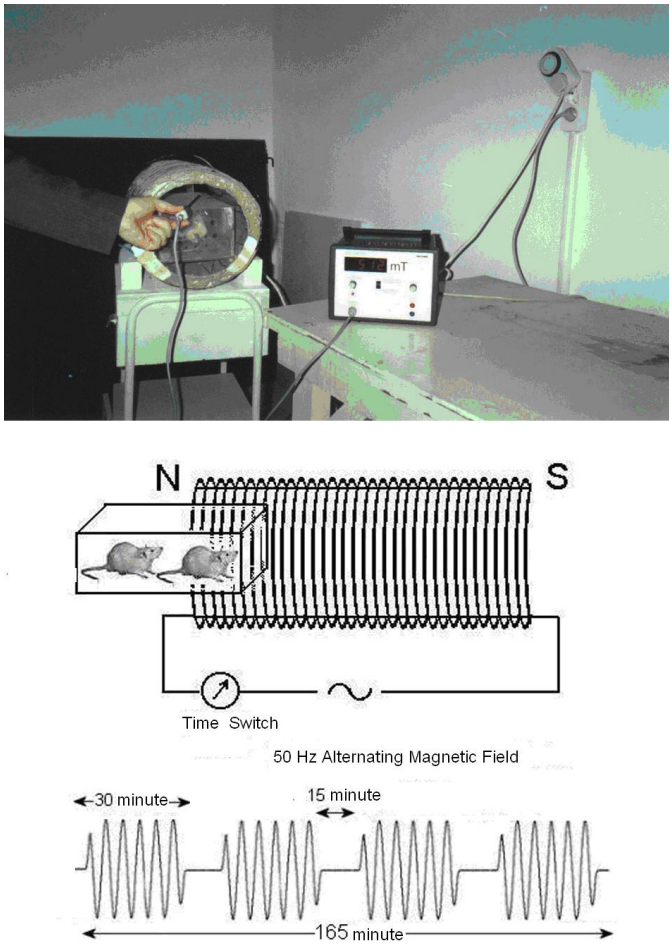

Fig. 1. A picture and scheme of solenoid used for rat exposure to sinusoidal magnetic field (50 Hz, 5 mT)

Determination of plasma zinc and copper levels: Plasma zinc and copper levels were determined by flame atomic absorption spectrophotometry following appropriate dilution with deionized water[19]. Calibration standards were prepared and analyzed in the same way. Readings were performed with Unicam-929 atomic absorption spectrophotometer at wavelength of 324.8 nm and 213.9 nm for copper and zinc, respectively.

Measurement of Na^+-K^+ ATPase activity: Assays were carried out in a final volume of 2.5 mL containing 0.3 mg tissue protein as the enzyme source (in the case of Na^+-K^+ ATPase activity measurements incubation medium contains 6 mM $MgCl_2$, 5 mM KCl, 100 mM NaCl, 0.1 mM EDTA, 30 mM tris-HCl buffer pH 7.4). After preincubation for 5 min at 37 °C, Na2ATP was added to each tube in a final concentration of 3 mM and the reaction was stopped by putting the samples on ice

after 0.5 h incubation with substrate. Na^+-K^+ ATPase and Ca^{2+} ATPase activities were assayed as the release of inorganic phosphate (Pi) from ATP according to the method of Atkinson *et al.*[20]. Protein contents of the samples were determined by the method of Lowry *et al.*[21].

Statistical analysis: Na-K ATPase of diaphragmatic muscles, plasma MDA, Cu and Zn levels were determined. Data were presented as mean ± SD. These data were analyzed with two-way analysis of variance followed by post hoc-Tukey test for pairwise comparisons. P values < 0.05 were considered statistically significant. All computations for the statistical analysis were carried out in SPSS program (SPSS, version 14.0, 2006; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Fig. 2 displays the plasma Cu levels of the study groups. The plasma Cu level of the DM-MFE group was significantly higher than those of the control, DM and MFE groups ($p < 0.05$). The plasma Cu level of the control group was significantly lower than those of the DM and MFE groups ($p < 0.05$).

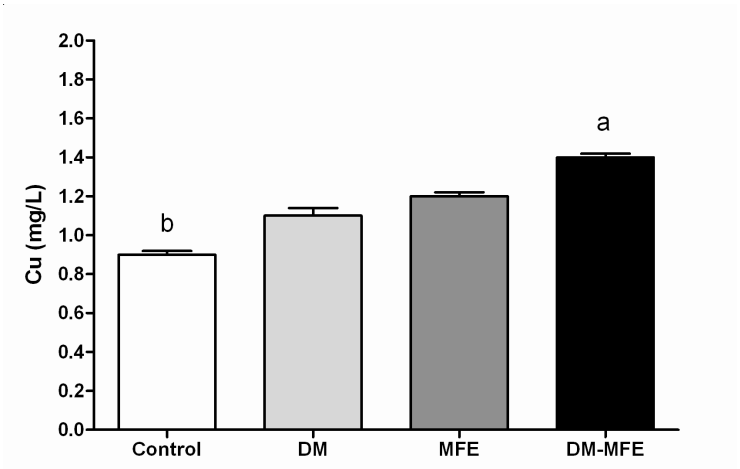

Fig. 2. Na-K ATPase of diaphragmatic muscle in control, DM, MFE and DM-MFE groups. Data were expressed as mean ± SD. DM, diabetes mellitus; MFE, magnetic field exposed and DM-MFE, DM plus magnetic field-exposed. [a]$p < 0.05$ *vs.* DM-MFE

Fig. 3 shows the plasma Zn levels in the study groups. The plasma Zn level of the DM-MFE group was significantly lower than those of the control, DM and MFE groups ($p < 0.05$). The plasma Zn levels of the DM and MFE groups were similar with that of the control group ($p < 0.05$).

Fig. 4 presents the plasma MDA levels in the study groups. The plasma MDA level of the DM group was significantly higher than those of the control, MFE and DM-MFE groups ($p < 0.05$). The plasma MDA levels of the MFE and DM-MFE groups were similar with that of the control group ($p < 0.05$).

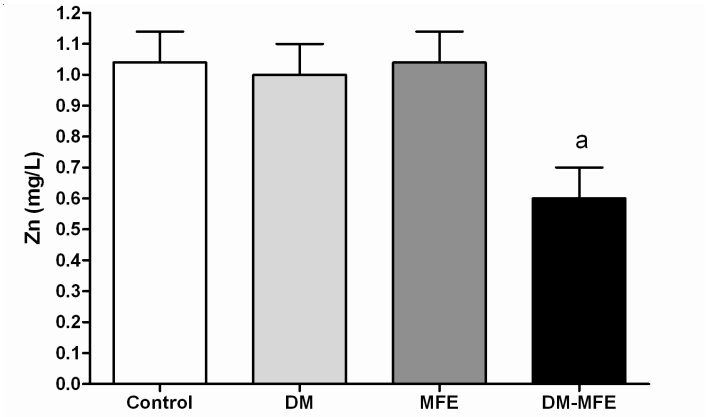

Fig. 3. Plasma MDA levels in control and DM, MFE and DM-MFE groups. Data were expressed as mean ± SD. DM, diabetes mellitus; MFE, magnetic field exposed; and DM-MFE, DM plus magnetic field-exposed. [a]$p < 0.05$ *vs.* Control, MFE and DM-MFE. [b]$p < 0.05$ *vs.* DM

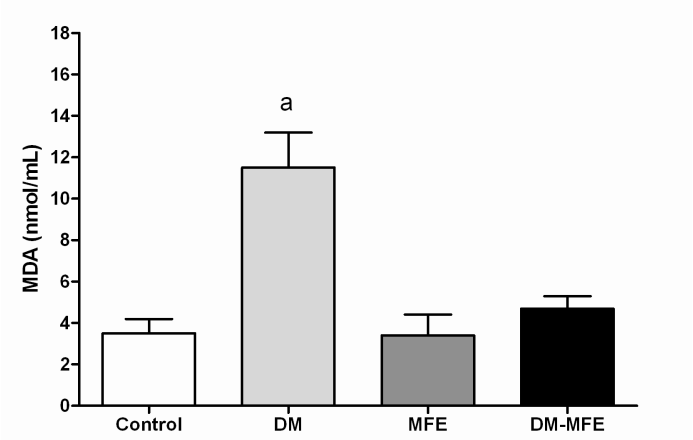

Fig. 4. Plasma Cu levels in control, DM, MFE and DM-MFE groups. Data were expressed as mean ± SD. DM, diabetes mellitus; MFE, magnetic field exposed; and DM-MFE, DM plus magnetic field-exposed. [a]$p < 0.05$ *vs.* control, DM and MFE. [b]$p < 0.05$ *vs.* DM and MFE

Fig. 5 shows the Na-K ATPase levels of diaphragmatic muscles in the control, DM, MFE and DM-MFE groups. The Na-K ATPase level of diaphragmatic muscles in the DM group was significantly lower than those of the control, MFE and DM-MFE groups ($p < 0.05$). The Na-K ATPase levels of diaphragmatic muscles in the MFE and DM-MFE groups were similar with that of the control group ($p < 0.05$).

This study examined the effect of MF on the Na-K ATPase level of isolated diaphragmatic muscle and plasma MDA, Cu and Zn levels in a diabetic rat model. Both MF and DM increased the plasma Cu level as compared to placebo and MF plus DM caused more increase in its level as compared to MF and DM. MF plus DM decreased the plasma Zn level as compared to placebo, MF and DM. MF did not cause any effect on the plasma MDA level of normal rats, but it decreased its

level of diabetic rats. MF did not cause any effect on the Na-K ATPase level of the normal diaphragmatic muscle, but it enhanced its level of the diabetic diaphragmatic muscle.

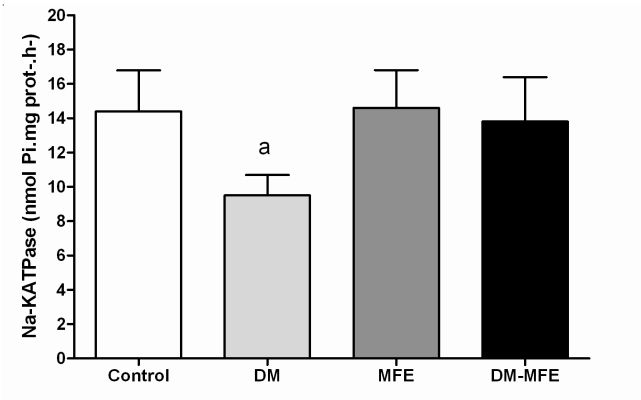

Fig. 5. Plasma Zn levels in control, DM, MFE and DM-MFE groups. Data were expressed as mean ± SD. DM, diabetes mellitus; MFE, magnetic field exposed and DM-MFE, DM plus magnetic field-exposed, $^{a}p < 0.05$ *vs.* control, DM and MFE

In this study, level of Cu increased in diabetic rats exposed to MF. The decrease of MDA level following EMF application could be explained by increased Cu level in diabetic rats exposed to MF. We hypothesized that EMF exposure could be followed by antioxidant production in diabetic rat tissues, because Cu-Zn SOD is an antioxidant enzyme. Therefore, increased Cu may affect SOD activities and increased SOD activity may decrease MDA level in diabetic rats exposed to MF. The plasma MDA level was higher in diabetic rats than rats of the other groups, however its level in rats exposed to MF were similar with control rats. Lipid peroxidation occurs at the cell membrane by the effect of free radicals[3]. MDA, the end product of lipid peroxidation, is used as a marker of oxidative stress[5]. Many authors have found increased levels of lipid peroxidase during diabetic disease[22,23]. However, Vessby *et al.*[11] did not find any difference between MDA levels of control and diabetic group. Lipid peroxidation is a well-established mechanism of oxidative damage caused by reactive oxygen species (ROS) and the measurement of the MDA provides a convenient index of lipid peroxidation[24,25]. Reports showing that electromagnetic field also increases concentrations of free radicals, lengthens their lifespan and enhances the probability that they can do damage to the body[26-29]. Present results show that MF didn't alter any changes on levels of MDA in control rats, although the level of MDA in diabetic rats exposed to MF decreased more than that of diabetic rats.

Zinc deficiency is one of the most important symptoms of DM. Because it is clear that the predominant effect on zinc homeostasis of DM is hypozincemia which may be the result of hyperzincuria or decreased gastrointestinal absorption of zinc or both[13]. According to present results, level of Zn in diabetic rats exposed to MF decreased when it compared with that of diabetic rats.

Copper is generally stored by in hepatocytes and Zn is found in several type of cells[30,31]. In present study, MF increased plasma Cu level and this may be explained by possibly increased release of stored Cu from cells. Furthermore, Laitle-Kobierska *et al.*[32] demonstrated that release of insulin can be triggered by MF in pancreatic β cells of healthy rats. In present study, contrary to the increase of Cu level, Zn level was decreased in diabetic rats exposed to MF.

Na^+-K^+-ATPase or Na^+-K^+ pump generates trans membranous Na^+-K^+ gradient and is essential for the specific properties of muscle such as contractility and excitability. DM is associated with a number of metabolic disorders, including impaired protein synthesis and increased protein degradation, that might influence the Na^+-K^+ pump concentration or Na^+-K^+-ATPase. Kjeldsen *et al.*[33] showed that DM decreased the Na^+-K^+-ATPase concentration of diaphragm muscle. This study provides evidence of a decrease in Na^+-K^+-ATPase concentration of diaphragmatic muscle induced by hyperglycemia. Emre *et al.*[34] determined that MF increased effect on Na-K ATPase of normal diaphragmatic muscle. However, there is no difference between the Na^+-K^+ ATPase concentrations of controls and MFE rats in present studies. On the other hands, MF enhanced Na^+-K^+-ATPase concentration of diabetic rats. Due to that, MF may change glucose level in blood *via* enhancing insulin. Laitle-Kobierska *et al.*[32] reported that MF enhanced releasing insulin from β-cells of pancreas of healthy rats. Enhanced insulin regulated on homeostasis of carbohydrate metabolism. Like that MF may be enhancing level of insulin in blood. According to some authors[35], the influence of ELF MFs on carbohydrate metabolism and the secretion of pancreatic hormones may be indirectly related to activation of the parasympathetic part of the vegetative nervous system, which is one of most significant factors stimulating hormonal activity of pancreas. Peroxidation of membrane lipids resulted in changes in fluidity and permeability, which could also affect functioning of membrane proteins[36]. As a matter of fact, the result even could change Na^+-K^+ ATPase activity.

In summary, in a diabetic rat model, although DM and MF increase plasma Cu level, MF plus DM have synergistic effect on it. While DM and MF do not affect plasma Zn level, MF plus DM decreases it. While DM increases plasma MDA level, MF decreases its level to normal. Although DM decreases Na-K ATPase of rat diaphragmatic muscle, MF elevates its level to normal. Interestingly, to exposure MF on diabetic rats minimizes the adverse effect of DM. DM increases plasma MDA level, the end-product of lipid peroxidation, in our laboratory settings and MF causes a beneficial effect with a decrease in adverse effect of DM on MDA level. This effect of MF may be explained by an increase in SOD activity related to an increase of Cu level by exposure to MF. In diabetic subjects exposed to MF because of working conditions such as transformer stations, further studies are required to determine possible beneficial effect of MF on clinical adverse effects of DM.

REFERENCES

1. A.R. Liboff and K.A. Jenrow, *NeuroRehabilit.*, **17**, 9 (2002).
2. C.B. Grissom, *Chem. Rev.*, **95**, 3 (1995).
3. J.C. Scaiano, F.L. Cozens and J. McLean, *Photochem. Photobiol.*, **59**, 585 (1994).
4. J.C. Scaiano, *Photochem. Photobiol.*, **62**, 818 (1995).
5. Z.A. Ovechkina, V.S. Martyniuk, S.B. Martyniuk and N.B. Kuchina, *Biofizika*, **46**, 915 (2001).
6. G. Ozdemir and F. Inanc, *Tohoku. J. Exp. Med.*, **206**, 247 (2005).
7. A.A. Shaheen and A.A. El-Fattah, *Int. J. Biochem. Cell Biol.*, **27**, 89 (1995).
8. M. Atalay and D.E. Laaksonen, *J. Sports Sci. Med.*, **1**, 1 (2002).
9. R.D. Bonnefont, J.P. Bastard and M.C.D. Jaudon, *Diabetes Metab.*, **26**, 163 (2000).
10. I.C. West, *Diabetic Med.*, **17**, 171 (2000).
11. J. Vessby, S. Basu, R. Mohsen, C. Berne and B. Vessby, *J. Int. Med.*, **251**, 69 (2002).
12. M.V. Aguilar, J.M. Laborda, M.C. Martinez-Para, M.J. Gonzalez, I. Meseguer, A. Bernao and C.J. Mateos, *J. Trace Elem. Med. Biol.*, **12**, 155 (1998).
13. J. Brandao-Neto, C.A.B. Silva, A.A. Rezende, M.G. Almeida, V.S.P. Sales and J.S. Marchini, *Nutr. Res.*, **23**, 141 (2003).
14. A.B. Chausmer, *J. Am. Nutr.*, **7**, 109 (19981).
15. Z.J. Hagay, Y. Weiss, I. Zumsan, M. Peled-Kamar, E.A. Reece and U.J. Erickson, *Am. J. Obst. Gynecol.*, **173**, 1036 (1995).
16. M.J. Salgueiro, N. Krebs, M.B. Zubillaga, R. Weill, E. Postaire, A.E. Lysionek, R.A. Caro, T. De Paoli, A. Hager and J. Boccio, *Biol. Trace Elem. Res.*, **81**, 215 (2001).
17. S.G. Kelsen and M.L. Nochomovits, *J. Appl. Physiol.*, **53**, 440 (1982).
18. H.N.O. Ohkawa and K. Yagi, *Anal. Biochem.*, **95**, 351 (1973).
19. A. Taylor, in ed.: S.J. Haswell, Clinical Applications of Flame Techniques, Atomic Absorption Spectrometry, Theory Design and Applications Elsevier, Amsterdam, pp. 359-379 (1991).
20. A. Atkinson, A.D. Gatenby and A.G. Lowe, *Biochim. Biophys. Acta*, **320**, 195 (1973).
21. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Rondal, *J. Biol. Chem.*, **193**, 265 (1951).
22. R.A. Anderson, A.M. Roussel, N. Zouari, S. Mahjoub, J.M. Matheu and A. Kekreni, *J. Am. Coll. Nutr.*, **20**, 212 (2001).
23. Y.R. Sailaja, R. Baskar and D. Saralakumari, *Free Radic. Biol. Med.*, **35**, 133 (2003).
24. S.K. Jain, T.K. Basu, N.J. Temple and M.L. Garg, Oxidative Stress, Vitamin E and Diabetes, Antioxidants in Human Health and Disease, UK, CABI Publishing, pp. 249-257 (1999).
25. F. Nielsen, B.B. Mikkelsen, J.B. Nielsen H.L. Andersen and P. Grandjean, *Clin. Chem.*, **43**, 1209 (1997).
26. H.V. Brezitskaia and O.I. Timchenko, *Radiats Biol. Radioecol.*, **40**, 149 (2000).
27. R.W. Eveson, C.R. Timmel and B. Brocklehurst, *Int. J. Radiat. Biol.*, **76**, 1509 (2000).
28. T. Yoshikawa, M. Tanigawa, T. Tanigawa and A. Imai, *Pathophysiology*, **7**, 131 (2000).
29. M. Zmyslony and J.M. Jajte, *Med. Pr.*, **49**, 177 (1998).
30. E. Mocchegiani, R. Giacconi and M. Malavolta, *Trends Mol. Med.*, **14**, 419 (2008).
31. P. Zatta and A. Frank, *Brain Res. Rev.*, **54**, 19 (2007).
32. A.L. Laitl-Kobierska, G. Cleslar, A. Sieron and H. Grzybek, *Bioelectromagnetics*, **23**, 49 (2002).
33. K. Kjeldsen, H. Braendgaard, P. Sidenius, J.S. Larsen and A. Norgaard, *Diabetes*, **36**, 842 (1987).
34. M. Emre, I. Gunay, G. Logoglu and T. Isbir, *Bioelectromagnetics*, **16**, 147 (1996).
35. V. Hefco, E. Hefco and C. Birca, *Rev. Roum. Biol-Zoologie.*, **14**, 79 (1969).
36. B. Balabanli, Y. Kamisaki, E. Martin and F. Murad, *Proc. Natl. Acad. Sci. (USA)*, **96**, 13136 (1999).

(*Received*: 16 April 2009; *Accepted*: 15 September 2009) AJC-7891