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EFFECT OF TECHNICAL SPORTIVE LOADING UPON THEMALONDIALDEHYDE (MDA) AND TRACE METALS LEVELS OF BOXERS

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Abstract
This study was carried out to investigate the effect of 2 hour technical boxing training upon antioxidant Malondialdehyde (MDA) and trace metals (Zinc – Selenium) levels. The study was participated by 30 male boxers from different weights with an average age of 20.09 ± 1.22 years. The participants were fed with a zinc and selenium free diet before the study and they were asked not to use and Zn- Se containing vitamins or tablets. There were 5cc venous blood samples taken from the participants and the participants were subjected to 2 hour training program after a 15 minute resting period. They 5 cc venous blood samples were taken from the participants at exhaustion. The Zn-Se and MDA levels were determined by ICP and Conti method respectively. The comparison of the pre and post training MDA and Zn-Se values revealed that there was a statistically significant difference between them ($p<0.01$). The results showed that the boxing training resulted a decrease in both the MDA and Zn-Se levels in blood. There was a positively directed weak correlation observed between post training MDA, Zn and Se levels which gave no significant difference.
Keywords: boxers, technical, sportive.

Introduction

Zinc is a micro nutrient necessary for more than 300 enzymes in the body. Zinc takes an important role in many metabolic processes and regulates the hormone balance of the body. It is also important for immune system, productive system, wound healing, skeletal development and intestinal functions [16] .Zinc status has an important effect upon physical performance. Athletes may have a zinc deficiency induced by poor diet and loss of zinc in sweat and urine. Limited data exist on the relationship of performance and zinc status (M. Hazar. 2009)

Selenium is an essential micro nutrient for the human body needed in trace amounts. It attaches to the proteins in order to form selenoproteins, a class of important anti oxidants. The antioxidant property of selenoproteins prevents the harm made by free radicals on cell membrane. Free radicals are the side products of oxygen metabolism and promote chronic diseases such as cancer and heart problems. Other selenoproteins take role in the regulation of the thyroid function and immunization system (M Conti., P.C.Morand, P. Levillain, A.Lemonnier, 1991). The biological active form of selenium is its organic form. Inorganic selenium is rapidly discharged from the body. In nature the plants convert selenium in to its organic form. Selenium is one of the minerals which show the most rapid decrease. In his article "Selenium .It is time to act "published in British Medical Journal in 1997 M.P. Rayman states that there has been a 50 % decrease in the level of Se taken from food in last 22 years. He also emphasizes the increase in cancer, heart diseases and impotency during the same period. The people with irregular feeding habits or cigarette smokers are particularly at risk because of selenium deficiency. Selenium deficiency is particularly effective in weakening of the immunization system. The daily recommended intake of Selenium is 55 µg [1]. Intensive exercises require excessive oxygen use and consequently increases the amount of free radicals formed. The body responds it by activating the antioxidant mechanism and administration of antioxidants in pain treatment may be employed to decrease the doses of analgesics (P.M. Clarkson 1991; H. Çakir, Farkli Şiddette Uygulanan, 2006).

Malondialdehyde (MDA) is a product formed by the peroxidation of polyunsaturated fatty acids . MDA forms as a result of peroxidation of the fatty acids containing three or more double bonds. These fatty acids in mammalians are mainly aracidonic and docosahegzanoic acids. The oxidation of oleic and linoleic acids result in the formation of lesser amount of MDA .The resulting MDA effects the ion exchange mechanism in the cell membrane and cross links the compounds on it thus changing the ion permeability and ion activity . Athletes are capable to discharge MDA in rapid manner as a result of regular exercises. However the increase in the rate of oxygen use may result the reactive oxygen species to overcome the immunization barrier and result in oxidative stress (P.M.Clarkson, 1991).This study revealed that acute loading resulted no statistically significant changes in MDA content of athletes. This may be explained with the fact that the boxers are capable of coping with oxidant species as a result of the adaptation they developed against acute sportive loadings.

The athletes as a result of regular training are able to remove MDA from the cells in a rapid way. The adoption against the resulting oxidative products, formed as a result of intensive training, developed with intensive exercises may be responsible for the chronically low MDA levels observed in athletes(P.M.Clarkson, 1991).

This study revealed that here was a significant change in the MDA levels as a result of acute exercise. This may be attributed to the high level of adaptation which the boxers developed against oxidative stress.

This study was carried out to investigate the change of the level of oxidant product MDA and anti oxidant Zn-Se as result of 2 hour intensive boxing exercise and to elucidate any relation between them.

Materials and methods

Physical and physiological measurements

Measurement of age, height and weight

Age of the participants is given in years. Height of the participants was measured with a metric scale on bare feet. The body weights were determined on bare feet with only shorts on with a Nan brand scale with an accuracy of ± 0.01 kg. All the measurements were taken one day before the start of the study.

Resting Heart-rate: The resting heart beat rates of the participants were recorded with a stethoscope at the morning of the day before the study at sitting and resting position. The results are given as beat per minute

Taking blood samples

There were 5cc of venous bloods taken from the left arms of the participants with heparinzed syringes by an expert nurse at rest position before the start of the training. The blood routes were kept open with a cut down catheter sealed with a plaster. The participants were allowed to rest for 15 minutes and subjected to 2 hours technical boxing training. There were 5 cc bloods collected from the participant at exhaustion just after the end of the exercise. The blood samples had been coded, centrifuged and kept at refrigerator before they were used for trace metal analyses.

Experimental method

ICP- OES Protocol microwave digestion procedure

Zinc and Se analyses were carried out in the laboratories of Science Faculty of Gazi University. Blood samples were drawn from the antecubital vein of the subject's right before, immediately after and I5 minutes after exercise. Sample preparations and measurements: On the I mL blood samples was added 2.0 mL HNO.i and the samples were digested in Berghof/Microwave Digestion system MWS-3 microwave apparatus. The microwaves were kept at 160 °C for 5 min and at 190, 100 and 80 °C for 10 min each. The totally digested samples were diluted to 10 mL with the addition of deionized water 18.3 18.3 mohm cm^{-1}.Zinc was analyzed directly using inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer, Optima 5300 DV, USA).There were 100,250,500 and 750 ug/L standard Se solutions were prepared from its 1000 ppm standard solutions and a calibration curve was plotted. Then selenium analyses of each sample were performed by making at least five readings. The results are tabulated in Table 2 Selenium was converted to its hydride before the analyses . 1 mL of 10% HCL was added onto 1 mL of digested blood samples and kept at 90 °C for 20 minutes. The samples were analyzed with the use of Perkin Elmer Optima 5300 DV model ICP-OES after they were cooled down.

Malondialdehyde (MDA) analysis

MDA measurements were carried out in the laboratories of Faculty of Medicine of Gaziantep University. Plasma lipid peroxidation was evaluated by a fluorometric method based on the reaction between MDA and thiobarbituric acid (TBA) (Conti Method). Briefly, 50 μL of plasma was added to 1 mL of 10 mmol/L diethylthiobarbituric acid (DETBA) reagent in phosphate buffer (0.1 mol/L, pH 3), mixed for 5 s and incubated for 60 min at 95 °C. Samples were placed in ice for 5 min and then 5 mL of butanol were added. The mixture was shaken for 1 min to extract the DETBA–MDA adduct, and then centrifuged at 1500 × g for 10 min at 4 °C. Fluorescence of the butanol extract was measured at the excitation wavelength 5

39 nm and the emission wavelength of 553 nm. 1, 1, 3,3-Tetraethoxypropane(Sigma) was used as a standard solution and the values were presented in μmol/L. [3]

Training protocol of the participants

Intensity of loading: 70 – 90 % sub maximal

Time: 2 hours

Resting: 5 minutes; for exercise 3 – 7 (Stretching)

1 - 15 minutes warm up (running)

2 - 15 minutes warm at rest (stretching)

3 - 5 x 3 minutes shadow boxing (5 round x 3 minutes)

4 - 5 x 3 minutes bag beating.

5 - 5 x 3 minutes modeling (Direct, uppercut, swing)

6 - 5 x 3 minutes sparring

7- 10 x 2 minute's lapa

8 - 10 minutes stretching

Statistical analyses of the data

The analysis of the data obtained was carried out by the use of SPSS 10.0 statistical software. The comparison of the pre and post training measures was made by paired simple t-test and the relations between two variables were evaluated by the use of Pearson Correlation technique.

Results

Physical parameters of the participants

Table 1. Physical parameters of the participants.

Parameter	Mean values ($\bar{X}$)	SS	Minimal Maximal
Age (year)	20.09	1.22	18 - 22
Height (cm)	176.00	6.67	163- 188
Body weight (kg)	72.45	15.37	48- 101
Resting heart rate (Beat/minute)	77.72	7.07	68 - 90

Pre and post exercise blood zinc levels of the participants

Table 2. The statistical analysis revealed that there is a significant difference between the pre and post- training Zinc values of the participants [$t_{(29)}$ =6. 23; p<0.01].

Element	N	Pre test mean value ($\bar{X}$)	S	Post test mean value ($\bar{X}$)	S	$\bar{X}1-\bar{X}2$	t	p
Zinc (µg/L)	30	245,64	15,70	216,70	10,29	28,94	6,23	.000

Pre and post exercise blood selenium levels of the participants

Table 3. The statistical analysis revealed that there is a significant difference between the pre and post- training Se values of the participants [$t_{(29)}$ =11.41; p< 0.01].

Element	N	Pre test mean value ($\bar{X}$)	S	Post test mean value ($\bar{X}$)	S	$\bar{X}1-\bar{X}2$	t	p
Selenium (µg/L)	30	277.18	1,15	208.42	5,78	68.76	11.41	.000

Pre and post exercise blood MDA levels of the participants

Table 4. The statistical analysis revealed that there is a significant difference between the pre and post- training MDA values of the participants [$t_{(29)}$ =8.13; p< 0.01].

Enzymes	N	Pre test mean value ($\bar{X}$)	S	Post test mean value ($\bar{X}$)	S	$\bar{X}1-\bar{X}2$	t	p
MDA (micromol/L)	30	4.99	1.16	3.47	0.99	1.52	8.13	.000

Correlation between pre test Zn and MDA levels

Table 5. There is a low level negative directed significant correlation between the pre test MDA and Zn levels of the participants [r=-0.329; p>0.05].

		Pre test Zn	Pre test MDA
Pre test Zn	Pearson Correlation	1	-,329
	Sig. (2-tailed)	.	,076
	N	30	30

Correlation between post- test Zn and MDA levels

Table 6. There is medium level positive directed significant correlation between the post test MDA and Zn levels [r=-0.244; p> 0.05].

		Post test Zn	Post test MDA
Post test Zn	Pearson Correlation	1	,244
	Sig. (2-tailed)	.	,193
	N	30	30

Correlation between pre test Se and MDA levels

Table 7. There is a medium level negative directed significant correlation between between the pre test MDA levels of the participants [r=-0.504; p< 0.05].

		Pre test Selenium	Pre test MDA
Pre test Selenium	Pearson Correlation	1.000	-.504
	Sig. (2-tailed)	.	.005
	N	30	30

** Correlation is significant at the 0.01 level (2-tailed).

Correlation between post- test Se and MDA levels

Table 8. There is a low level positive directed week an in significant relation between the post test MDA and Se levels [r=-0.202; p> 0.05].

		Post test Selenium	**Post test MDA**
Post test Selenium	Pearson Correlation	1.000	-.202
	Sig. (2-tailed)	.	.285
	n	30	30

Discussion

Burn et al. have investigated the serum zinc levels of 20 gymnasts (9 males and 11 females) and found that the average serum zinc level was 0.599±0.026 mg/L which was below the value obtained 116 sedentary control group (0.81±0.014 mg/L).The average serum zinc level he level of girls (0.557±0.023 mg/L) was found to be lower than that of boys (0.651±0.044 mg/L). These values were found to be significant at (p<0.01) level. There was also a positive correlation between the isometric adductor muscle strength and blood zinc level (p<0.05).

Cordova et al.studied the blood zinc levels of 12 volleyball players and 12 controls and found that there was an increase in both of them after the exercise. They also observed that 24 h zinc discharge with urine showed a 22% increase in volleyball players and showed a slight decrease in the control group. There was an enormous increase in the discharge of zinc with sweat in volleyball players (300%) as a result of prolonged exercise while this value remained 30% for the control group. The serum zinc levels of athletes increased by 4% in athletes and 2% in controls. Van Loan et al (J.F.Run, C.Dieu-Cambrezy, A.Charpiat C.Fons, C.Fedou, J.P.Micallef, M.Fussellier, L.Bardet A.Orsetti) nvestigated the effect of zinc loss upon the muscle functions and they found that plasma zinc level showed a decrease of 67% as a result of isokinetic extension. They also observed that the loss of zinc caused a significant decrease in muscle strength and total work capacity.

There is no consensus about the status of the zinc level with the physical exercise while others say the opposite. Some of the researchers claim that the blood zinc levels decrease. Kaya (F.Rslan,2009), A. Hazar (Ikukawa., A. Kobayashi., 2002), Aslan (An Loan Md., B. Sutherland, N. Lowe., J.R.Turnlund ., JC.King, , 1999), Van Loan et al (J.F.Brun, C.Dieu-Cambrezy, A.Charpiat, C. Fons., C. Fedou., JP. Micallef, M,Fussellier, L.Bardet, A. Orsetti A,1995).Kikukawa and Kobayashi This study showed that Zinc level decreased from 245.64 ± 15.70 µg / L to 216.70 ± 10.29 µg /L as a result of acute training.

As an integral part of glutathione peroxydase and tioredoxine reductrase selenium probably interacts with each compound which effects the antioxidation balance. Among the critical components of the antioxidant enzymes are copper, zinc (as süperoxide dismutase) and iron (catalase). Selenium acts in the limitation of the oxidation of fats and supports the activity of vitamin E as glutathione peroxidase The experiments carried out on animals showed that selenium and vitamin E complement each other and revealed that selenium may prevent some of the complications stem from the deficiency of vitamin E (M Conti., P.C. Morand., P.Levillain, A.Lemonnier, 1991).Few results are available concerning Se concentration during or following physical activity (M. Jose., L. Alonso., A. B. Barrera., J.´ A. Cocho, De J., Jose´ M. F. Bermudezb., P. B. Barrera., 2005; Ga Milias., T. Nomikos, E. Fragopoulou, S. Athanasopoulos, S. Antonopoulou, Biofactors, 2006).The selenium concentrations obtained for each samples are follows: For the human placenta between 0.56 and 1.06 µg/g , for the umbilical cord blood 51.1–104.2 µg/g , for the maternal blood between 57.3 and 117.9 mg/l and for hair and nails 0.22–1.5 µg/g and 0.46–1.57 µg/g (R. Kyta., V. Holecek.,I. Pekárková, J.Krejcová., J.Racek, L.Trefi,A.Yamamotová, 2003) Milias et al (H C. Ukaski., 2004)examined the effect of eccentric exercise upon baseline serum levels of selenium (Se), a trace element that participates in both antioxidant and anti-inflammatory systems, affects the overall response to injury. Thirteen males performed 36 maximal eccentric actions with the elbow flexors of the non-dominant arm on a motorized dynamometer. The main finding of this study was that baseline Se serum levels were associated inversely with CK, LDH and flexed arm angle (FANG) and positively with isometric torque (MIT), and range of motion (ROM) (p<0.05). These data suggest that beyond overt Se deficiency, suboptimal Se status possibly worsens muscle functional decrements subsequent to eccentric muscle contractions. This study showed that selenium level decreased from 277.18 ± 1.15 µg / L to 208.42 ± 5.78 µg /L as a result of acute training.

The literature results related to the MDA response towards an acute exercise are sporadic. There are reports claiming the MDA levels decreased, increased or unchanged after the exercise (H.Çakir, F. Ş. Uygulanan, 2006).

Athletes are able to discharge MDA from the cells in a rapid manner as a result of adaptation developed by regular exercise. This may explain the chronically low MDA levels observed in athletes after strenuous exercise (H.Çakir, F. Ş. Uygulanan, 2006).When we look at the studies related to the change of the anti oxidant protection mechanism we see that there is a decrease in MDA levels after sportive activities. The MDA levels were observed to show a statistically significant decrease as a result of acute exercise. The MDA value showed a decrease from 4.99 to 3.47 µmol/L. This is in perfect concordance with the studies of Çakır (2006) who found a statistically significant decrease at MDA levels at the end of the fourth week in two groups of participants subjected to a two different 6 week endurance training ($p<0.05$) N.Cases, A.Sureda, I.Maestre, P.Tauler, A.Aguilo , A.Cordova ,et al., 2006). However Cases et al [8].stated that there was no significant change in the MDA levels as a result of cycling races which took 235 and 255 minutes respectively in their study carried out on 177 cyclists ($p>0.05$) (8). Robertson et al. reported that the MDA levels of sedentaries were higher than the runners after an acute exercise in their study they carried out on 6 sedentaries and 20 runners making different exercises (margaritis i., tessier f., prou e., marconnet p., marini j-f., 1997). Aslan et al. (J.Pincemail, J.Lecomte, J.Castiau et al., 2000) applied a 15-20 minute sub-maximal running training protocol to 15 healthy male participants and observed a significant increase in the MDA levels after the training period ($p<0.01$). T.Sinoforoglu (reported that the MDA levels of the athletes did not show any significant difference after a 50 min aerobic ($p>0.05$). However there was a significant decrease in the sedentaries after the exercise ($p<0.01$). S.Gonenc (Jd. Robertson., Rj. Maughan, Gg Duthie., PC. Morrice., 1991) reported that there was a significant change in the MDA levels of children after 4 week swimming exercise ($p<0.05$).

This study also revealed that MDA levels decreased from 4.99 ±1.16 to 3.47 ± 0.99 µmol/L after the exercises which comply well with literature values.

There was not any significant difference in MDA values. This is due to the fact that regular exercising activates the anti oxidant defensive mechanism which increases the removal of MDA from the body. The main reason for the decrease in MDA levels in our study increased antioxidant defensive mechanism of the athletes as a result strenuous exercises. However as a result of increased oxygen utilization during sportive loading process which may surpass the anti oxidant defensive mechanism may eventually cause an oxidative stress. The decrease in MDA levels after the exercise may be attributed to the adaptation mechanism boxers developed.

In conclusion there was a significant decrease in Zn-Se and MDA levels as a result of acute sportive training ($p<0.01$). This may be explained by the quick adaptation of the boxers to the oxidative stress as a result of regular training. The decrease in Zn and Se levels may be attributed by the increased free radical production as a result of increased oxygen use during the physical activity which in turn activates the antioxidation mechanism.

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