Effect of acute maximal aerobic exercise upon the trace element levels in blood

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Abstract

This study was carried out in order to determine the effect of acute maximal aerobic exercise on the copper and zinc levels in blood. The study was participated by 16 healthy male university students with an average age of 19.44±1.63. There were 5 cc blood samples taken from the participants before and after they had been subjected to aerobic loading process (20 m shuttle run). The copper and zinc levels in the blood samples were determined by the use of Anodic Stripping Voltammetry (ASV) technique. The data obtained were evaluated by simple t test and SPSS software. The results revealed the fact that the blood Zn levels of the participants increased and Cu levels decreased with statistical significant extent ($p<0.01$) after maximal aerobic loading. There found no correlation between the maximal aerobic power levels (Max V_{O2}) of the participants and their resting copper and zinc blood levels. However blood zinc and Max V_{O2} levels of the participants were positively correlated after maximal aerobic loading. The participants were fed on a zinc and copper free diet six week prior to the program. They were also asked not to use copper and zinc containing vitamins during this period.

ORIGINAL ARTICLE

INTRODUCTION

Physical exercise leads to many metabolic, cardiovascular, and muscular changes in the body. The trace elements of zinc and copper are directly involved, as enzymatic cofactors, in many of these processes, especially those related to nutrients metabolism, oxygen transport, and creation of usable energy (Bordin *et al.*, 1993). 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 (Lukaski, 2004). 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 (Clarkson, 1991). Exercise increases zinc loss from the body, and dietary intake for some athletes, especially females, may be inadequate. Blood copper levels are altered by exercise, but there is no information to suggest that copper ingestion or status is compromised in athletes (Clarkson and Haymes, 1994). Gormüş *et al* (2005) investigated the relation between leptin levels as an acute response extracorporeal circulation and zinc and copper levels and found increased leptin levels, while zinc and copper levels showed a decrease. Kiliç *et al*. (2006) on the other hand claims that physiological doses of zinc administration may benefit performance.

The effects of high-intensity physical exercise on plasma levels of Cu^{2+} and Zn^{2+} in 19 subjects are investigated (9 males and 10 females). Plasma copper concentration was found to decrease and plasma zinc concentration increase after exercise in both sexes (Bordin *et al*., 1993).

Mineral elements, including magnesium, zinc, and copper, are required by the body in modest amounts for the maintenance of health and for the development of optimal physiological functions. For athletes, adequate amounts of these minerals are required for physical training and performance. Studies of athletes during training, as compared to non-training control subjects, indicated the potential for increased losses of minerals by sweat and urine (Lukaski, 1995).

There is no consensus about the status of the zinc level with the physical exercise. Some of the researchers claim that the blood zinc levels decrease (Deruisseau *et al*. 2002; Van Loan *et al*.,1999) with physical exercise while others say the opposite (Kikukawa and Kobayashi, 2002).

Copper is one of the most commonly found trace metals and is an essential micro nutrient for the body. Copper deficiency results in Menke disease and some hereditary disorders (Sports Medicine Manual, 1987). Copper is necessary for nearly 30 enzymes in the body. Its deficiency manifests itself as a decrease in plasma concentration. The studies indicated no correlation between the copper deficiency and the physical performance (Deruisseau *et al*., 2002; Kikukawa and Kobayashi, 2002). The purpose of this study is to investigate the effect of acute maximal exercise upon the blood levels of these trace elements. The correlation between Max VO2 level and blood levels of trace metals will also be elucidated.

MATERIAL AND METHOD

Selection of the Participants

The study was carried out on 16 male university student volunteers with an average age of 19.44±1.63 years. The participants were informed about the importance of the study in order to increase their motivation. They also read the signed the forms related to the regulations to be complied throughout the study. The physical and the physiologic features of the participants were determined. There were 5 cc venous blood samples taken from the participants after they had adequately rested (their pulse rate and blood pressures were monitored). They warmed up for ten minutes before they had 20 m shuttle run in a hall. There were 5 cc venous blood samples taken again right after the end of the test. The pulse rates of the participants were constantly monitored in order to determine whether they reached their maximal exhaustion levels (220 – Age).

Physical and Physiological Measurements

Measurement of height and weight

The heights and the weights of subjects were determined with an electronic scale with wearing no shoes having only a short on, a day before the start of the study.

Measurement of resting hearth rate

The resting hearth rate of each participant was measured in pulse/min at sitting position with stethoscope for 1 minute at the morning before the start of the study.

Determination Max VO_2 value

Max VO_2 values, measure of the cardiovascular efficiency and aerobic capacity of the participants, were measured as mL.kg/min with a 20 m shuttle run. The results were estimated from the Max VO_2 tables (Ramsbottom *et al*., 1988). The exhaustion levels of the participants were determined taking the hearth rates at the end of the study.

Collection of the blood samples

There were 5 cc venous blood collected from the left arms of the participants before the maximal loading process with plastic tip no metal containing heparinized plastic syringes at sitting position. The venous route was kept open with cut down catheter in order to take another 5 cc blood after the exhaustion process. The participants were subjected to 20 m shuttle runs in order to test their aerobic limits. There were 5 cc venous bloods collected from the participants again at their exhaustive state. The blood samples were centrifuged and kept in deep freeze.

Experimental

All the reagents used were in analytical grade. The solutions used for standard addition process were prepared from 0.1 M $Cu(NO_3)_2$ and $Zn(NO_3)_2$ (Merck) stock solutions. The buffer solution was prepared from acetic acid (Riedel de Häen) and NaOH (Merck). Nitric acid used for digestion process was in spectroscopic grade and the samples were prepared using de ionized water (16.8 MΩ)

Digestion of the samples

The digestion procedure was carried out in Berghof/ Microwave Digestion System MWS-3 speedwave apparatus after taking 1 mL of blood and adding 2.5 mL HNO_3 on it. The microwave was kept at 160 °C for five minutes, 190 °C, 100 °C and 80 °C for ten minutes each. The totally digested samples were diluted to 10 mL with the addition of deionized water (16.8 MΩ).

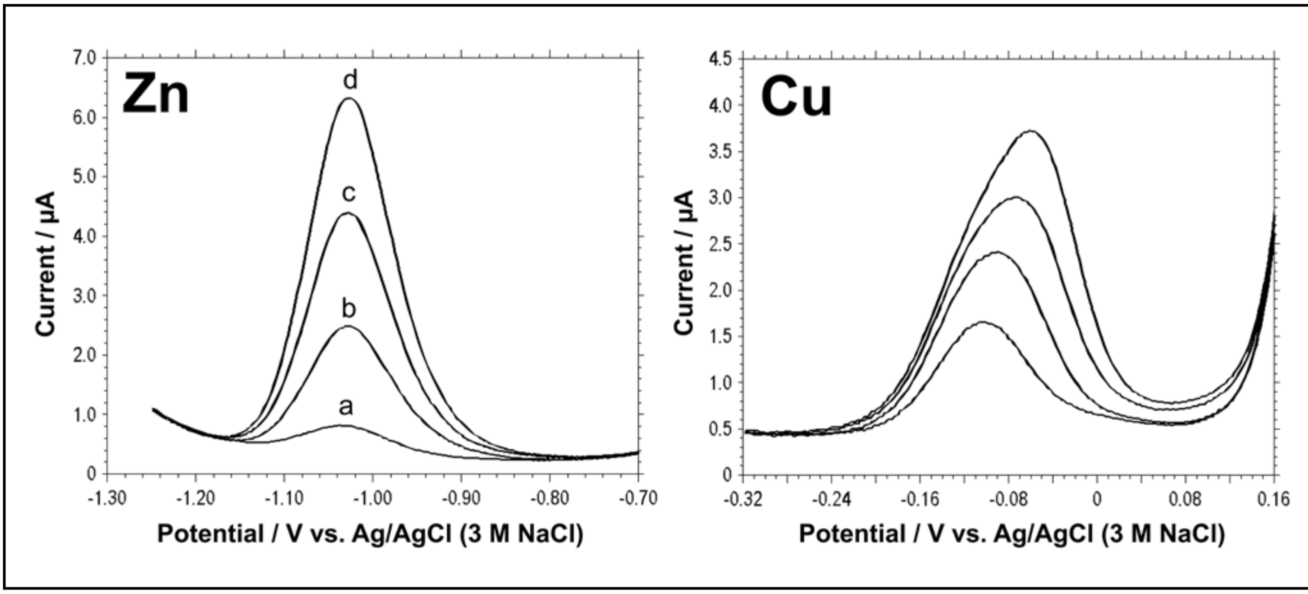

Figure 1. Anodic square wave voltammograms of Pb^{2+} peak located at –0.47 **a**) 0.5 mL sample + 2 mL acetic acid-acetate buffer **b**) Addition of 20 μL 10^{-4} M Cu^{2+} and Zn^{2+} **c**) Addition of 40 μL 10^{-4} M Cu^{2+} and Zn^{2+} **d**) Addition of 60 μL 10^{-4} M Cu^{2+} and Zn^{2+}.

Voltammetric Procedure

The trace elements analyses of the samples were carried out by the use of square wave stripping voltammetry under the conditions given in Table 1. The electrochemical analyses were performed computer controlled CHI660B model potentiostat. The working electrode was 100 μm capillary hanging mercury electrode (BAS CGME) and the counter and reference electrodes were a Pt wire and Ag/AgCl (3 M NaCl). The residual oxygen in the system was removed by purging Ar gas with spectrophotometric purity. The peak potentials for Cu^{2+} and Zn^{2+} were –0.10 V and –1.05 V (Ag/AgCl) under the conditions stated in Table 1 (Figure 1).

Analytic Procedure

0.5 mL of the samples which had been previously made up to 10 mL with deionized water was taken into the cell and 2 mL acetic acid- acetate buffer was added to it. The solution was stirred for 2 minutes before the stripping process. The final voltammogram is given in Figure 1. The voltammograms obtained after three standard additions of 20 μL 10^{-4} M Cu and Zn solutions were superimposed upon each other. The resulting voltammograms were displayed in Figure 1. The increase in the Cu and Zn peaks at –0.10 V vs. –1.05 V was evaluated according to standard addition method. The procedure was repeated three times for each sample and the average value of the amount of copper and zinc in each sample was computed. The blank study revealed that the amount of metal coming from nitric acid and de ionized was negligible. In any case the amount of metal found in the blank sample was subtracted from the amount obtained for the actual samples. The results are tabulated in Table 2 in μg Cu/dL blood and μg Zn/dL blood together with standard deviations.

The stripping analysis is based upon cathodic deposition of the related metal ion and the anodic stripping with a linear potential scan (Barendrecht, 1967; Neeb, 1969; Vydra *et al.*, 1977).

Table 1. The electrochemical analysis conditions of square wave stripping voltammetry.

Deposition potential	–1.250 V
Deposition time	150 s
Scan rate	2 mV
Amplitude	25 mV
Frequency	30 Hz
Rest time	15 s
Stirring rate	350 rpm

Table 2. Some of the physical and physiological parameters of the participants.

Parameters N=16	**Mean**	**SD**	**Min–Max**
Age (year)	19.44	±1.63	17–23
Height (cm)	183	±7.36	168–200
Body weight (kg)	76.06	±7.63	68–98
Resting Hearth Rate (RHR) (Beat/min.)	67.88	±3.63	60–72
Max VO_2 mL.kg/min	43.68	±4.15	36.81–51.30

Analysis of the data

Te descriptive statistics of the data, the t-test of significance between the pre and post tests results and the correlation between the trace metal levels and the Max VO_2 values of the participants were computed with the use of SPSS 10.0 software at a significance level of $p<0.01$.

RESULTS

It is observed that the blood zinc levels of the participants showed a statistically significant decrease after the maximal aerobic loading process (from 0.020±0.002 ppm to 0.011±0.003 ppm) while the blood copper levels exhibited a statistically significant increase (from 0.244±0.029 ppm to 0.370±0.032 ppm) $p<0.01$ (Table.3).

There was a positive correlation between the Max VO_2 values and the blood Zn levels of the participants after the maximal aerobic loading process ($p<0.05$). However there was no correlation between the Max VO_2 levels and resting zinc and copper and post maximal exercise copper values of the participants (Table 4).

DISCUSSION

Resting Hearth Rate (RHR) is closely related to the level of activity. Hearth rate is observed to decrease after training (Mc Ardle *et al.*, 1994; Sports Medicine Manual, 1987). Low hearth rate as a result of high stroke volume during sub maximal exercise reflects a good fitness level. It is known that endurance exercises result in the decrease of the resting and training hearth rate and increase stroke volume (Mc Ardle *et al.*, 1994; Sharkey,1990)

The mean resting hearth rate observed in this study was a little bit higher compared with that of elite sportsmen but similar to that of healthy people (Akova *et al.*, 2005).

Table 3. The comparisons of the average trace metal levels in the blood of the participants at resting and after the maximal aerobic loading.

Parameters	Zn level		Cu level	
N=12	Resting	After max. loading process	Resting	After max. loading process
Max VO_2	r=0.194 p=0.472	r=0.511* p=0.043	r=−0.137 p=0.614	r=0.070 p=0.796

*p<0.01

Aerobic power is described as the capacity of taking, transporting and utilizing oxygen.The best method to determine the aerobic capacity or fitness is the direct measurement of the oxygen utilization capacity (Max VO_2). However this method is both time consuming and requires sophisticated equipment. There were various field tests developed to measure Max VO_2 values (Dolgener *et al.*, 1994; Sharkey, 1990). Aerobic capacity is developed and maintained by the exercises which activate the hypertrophy of the muscles (walking, cycling, and swimming). Average Max VO_2 values are in the range of 4–5 L/min for the ordinary people. However this value may well reach to 5–6 L/min for the elite sportsmen. These values express the total capacity. However this capacity is also related to the size of the body. That is why Max VO_2 values are better to be stated in mL/min.kg (Sharkey, 1990). Darling *et al.* (2005) found the average Max VO_2 value 61.5±7.7 mL/min.kg in their study they carried out on 20 healthy male university students subjected to continuous running. The Max VO_2 value of 43.68±4.15 mL/min.kg obtained in this study is also within similar range with literature.

Zinc takes place in the function of nearly 300 enzymes. The determination of slight zinc deficiency is difficult since it gives no manifestation. Zinc deficiency causes anorexia, weight loss, loss of endurance and osteoporosis in the athletes (Micheletti *et al.*, 2001). The low serum zinc levels were reported to cause the increase in the viscosity of blood and decrease the physical performance (Khaled *et al.*, 1999).

Copper also takes role in the function 30 enzymes. However there found no correlation between the physical exercise and the copper levels in blood after maximal aerobic loading. Although some researchers claim that copper levels in blood increases with exercise (Deruisseau *et al.*, 2002) there is no consensus here as in the case of Zn. Resina *et al.* (1990) investigated the serum copper level of 41 elite athletes and 24 person control group stated that the serum copper concentrations were lower and plasma levels were higher in athletes. According to their study the mean serum copper level of athlete was 14.95 μmol/L compared to that of 19.77 μmol/L for the control group. Marrella *et al.* (1993) measured the pre and post competition copper and zinc plasma and total blood concentrations of 16 marathon runners and found that although plasma blood levels were normal there was 29.3% decrease in copper and 29.5% increase in zinc compared to the control group as regards to total blood concentrations. They stated that heavy

Table 4. Basic correlation values between the parameters.

Trace element (ppm)	Resting condition	SE	After Maximal Aerobic Loading	SH	X1–X2 (ppm)	t-value	p-value
Zn	0.020±0.002	0.001	0.011±0.003	0.001	0.009	−9.704*	0.000
Cu	0.244±0.029	0.07	0. 370±0.032	0.008	0.126	−16.429*	0.000

*p<0.05

exercises modified the heavy metal metabolism. Ohno *et al.* 1990 investigated the effect of exercise upon Zn and Cu levels of 7 sedentary students between 18–19 years of age. The students took no vitamin complexes throughout the study and their blood samples were collected after a resting period of a night before they eat anything. They were then subjected to 12 minute bicycle ergometric test. Their bloods were taken again after this test. It was observed that Max VO_2 value increased from 43.5±1.5 to 48.1±1.8 mL.kg/min ($p<0.01$). Plasma zinc level decreased from 79.7±2.7 to 78.0±3.1 µg/100 mL and total copper level decreased from 83.1±5.1 and 79.9±4.6 µg/100 mL. Brun *et al.* (1995) 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 ($p<0.05$). Lukaski *et al.* (1996) in their study they carried out on swimmers proved that copper zinc, magnesium and iron were effective on the performance. The blood Cu and Zn levels were found to be 15.9±1.1 and 12.7±0.1 µmol/L for female and 13.9±0.6 and 14.6±0.6 µmol/L for male swimmers. Cordova *et al.* (1998) 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.*, (1999) investigated 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. Kikukawa *et al.* (2002) in their study they carried out 11 air rescue operators working in the Japan army determined that the urine levels of both Cu and Zn increased as result of acute exercise. De Ruisseau *et al.* (2002) found that the amount of zinc discharged with sweat showed a decrease as a result of 2 h-heavy exercise in their study on 9 male and 9 female cyclists. Saraymen *et al.* (2003) investigated the sweat copper and zinc levels of 21 boxers and found that the average copper level was 37.7±5.4 µg/dL and the average zinc level was 44.4±5.9 µg/dL after 30 min exercise. Again Saraymen *et al.* (2004) found the corresponding values as 28.58±3.3 µg/dL and 42.6±4.0 µg/dL in wrestlers. The data obtained in this study are in good accordance with literature (Lukaski *et al.*, 1990).

In conclusion the blood zinc levels of the participants showed a decrease while the blood copper levels showed a statistically significant increase as result of acute exercise. There were no correlation between the aerobic power (Max VO_2) and resting copper and zinc blood levels of the participants. However it was observed that the blood zinc levels after maximal loading was positively correlated with Max VO_2 levels ($p<0.05$).

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