ORIGINAL ARTICLE

Effect of six weeks aerobic training upon blood trace metals levels

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Abstract

This study was carried out to investigate the effects of 6-week aerobic exercise program upon blood Zn and Cu levels. There were 12 male university students with an average age of 21.67 ± 0.89 years and no regular training habits participated in the study. The participants were subjected three days a week 1 hour a day continuous running program on treadmill with an intensity of 60–70% for a period of six weeks. They were fed with zinc and copper free diet throughout the study and it was made sure that they were not using copper or zinc containing vitamin tablets. The differences between the pre and post study periods were found to be statistically significant as regards to both resting and maximal loading conditions ($p<0.01$). The pre and post training maxVO2 values were also found to be positively correlated with the copper and zinc levels in blood. Both the copper and zinc blood levels were significantly decreased after 6-week aerobic training period $p<0.05$.

Introduction

Zinc is a micro nutrient necessary for more than 300 enzymes and takes important role in many metabolic processes such as nucleic acid and protein synthesis, cell propagation, utilization of glucose, reproduction, immunity, tasting, wound healing, skeletal development and intestinal functions [9]. Zinc status also has an important effect upon the physical performance. However there is no consensus regarding to the blood levels of zinc after the exercise. Some researchers claim that blood zinc level is depleted after the exercise [5, 22] while others claim the opposite [12]. Copper is also one of the essential micronutrients for the human body. Copper deficiency causes some hereditary diseases such as Menke disease [20].

Copper is present in more than 30 enzymes in the body. The studies revealed that that there is no correlation between the copper deficiency and the physical exercise taken. There is no agreement on that issue as in the case of zinc. However, it is though that large amount of copper ions is transferred to the drinking water from the copper ducts used to carry it. The vagueness in the amount of copper in blood before and the after the strenuous exercise may also be due to this fact.

The aim of this study is to contribute to the elimination of this vagueness in literature and determine the effect of 6 week aerobic exercise upon the concentrations of trace metals in blood and reveal any relation between the blood zinc or copper levels and maxVO2 values.

Material And Method

Selection of the participants

The study was carried out upon 12 male participants with an average age of 21.67 years ± 0.89 and an average height of 180 cm ± 6.07 who had no regular exercising habits who did sports only at fitness level. The participants were subjected to a 6 week aerobic exercise protocol with 1 hour runs on a treadmill three days a week. The participants were given adequate information about the importance of the study in order to motivate them to take part in it. They were also informed about the rules they had to obey throughout the study and each of them signed a voluntary participation form. After determining the physical and physiologic conditions of the participants they were subjected to some selected tests. Then 5cc of blood was taken from the participants at rest. The participants were subjected to 20 m shuttle runs after 15 minutes rest period to test their aerobic limits. The purpose of this was to tire the participants to the exhaustion. At the end of this test 5 cc blood was collected from the exhausted participants. Their heart beat rates, systolic and diastolic blood pressures were recorded before and after the test the test. These measurements were repeated in the same manner at the end of the six week training period.

Physical and physiological measurements and tests

Age, weight and height. The age of the participants were accurately recorded in years. The heights were measured without shoes using the metric plate of the NAN brand scale. The weights were measured with participants wearing only a short without shoes at an accuracy of ± 0.01 kg. These measurements were taken the day before the training session started and the day after the end of the 6 week exercise period.

Resting heart beat rate. The heart beat rate were taken at the morning of day before the start of the training session and the day after the end of the exercise period having the participants in sitting position for a period of 1 minute using a stethoscope and a chronometer.

Systolic and Diastolic Blood Pressure. The systolic and diastolic blood pressures of the participants were measured with the use Bosch brand mechanical sphygmanometer at sitting and resting position. This parameter was measured 1 day prior and 1 day after the 6-week training period.

20 Meter Shuttle run and max VO2 Determination. MaxVO2 values of the participants were determined with a 20 m shuttle run which shows the cardio respiratory efficiency and aerobic capacity. The results were estimated from maxVO2 evaluation tables [17].

The heart beat rates were measured at the end of the test in order to determine the exhaustion levels of the participants.

Collecting the blood samples. 5 cc of venous blood samples of the participants were taken at resting and sitting position from their left arms with the use of heparinized plastic syringes. The blood vessel was kept open with a cut down catheter. The participants were then exhausted to their aerobic limits with 20 m shuttle runs before extracting 5cc blood at exhaustion. The samples were labeled, centrifuged and kept in a deep freeze.

Experimental Procedure

Chemicals and solutions

All the chemicals employed in the digestion of the samples and the preparation of the solutions were of analytical grade. The stock solutions were prepared by ten times dilutions of 0.1 M Cu $(NO_3)_2$ and Zn $(NO_3)_2$ (Merck) solutions. The PH of the medium was adjusted to pH 4.70 with the acetic acid acetate buffer. All the solutions were prepared with the use of deionized water (16.8 MΩ). The blood samples were digested with the addition of HNO_3 (Merck).

Digestion of the samples

2.5 mL of HNO_3 was added upon 1 mL of blood samples and the samples were digested in microwave apparatus (Berghof/Microwave Digestion System MWS-3 speedwave). The microwave were kept at 160 °C for five minutes and at 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).

Voltammetric procedure

The trace amounts of Cu and Zn were determined with the use of anodic stripping square wave voltammetry using a computer controlled CHI 660B potentiostat, associated with BAS CGME hanging mercury drop electrode in a three electrode cell. The reference and counter electrodes were Ag/AgCl (3 M NaCl) and a Pt wire respectively. The solutions were purged with purified argon for ten minutes prior the experiment in order to remove the residual oxygen and blanketed thereafter. The experimental conditions were tabulated in Table 1. The reduction potentials of Cu2+ and Zn2+ were determined as −0.10 V and −1.05 V Ag/AgCl (3 M NaCl) (Figure 1).

Analytical procedure

0.5 mL of the digested sample was put into an experimental vial and 2mL of acetate buffer was added to them. The solution was stirred for two minutes and the resulting voltammograms are given in Table 2. The voltammograms obtained after the standard additions of 25 μL 10^{-4} M Cu and Zn solutions were superimposed upon each other.

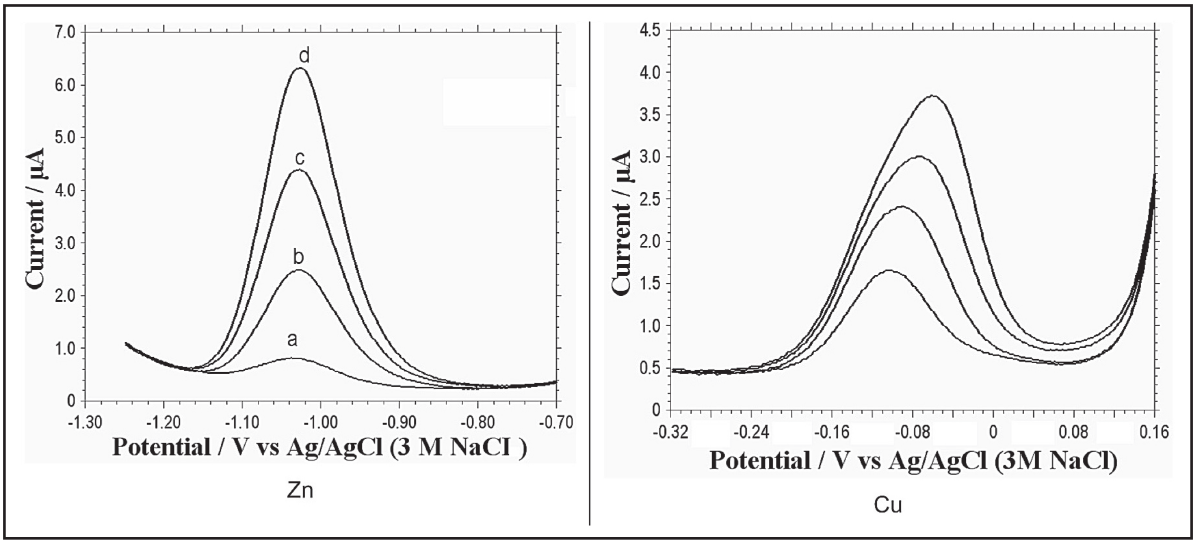

Figure 1. Square wave anodic square wave voltammograms of Cu^{2+} and Zn^{2+} peaks located at –0.10 V and –1.05 V Pb^{2+} **a)** 0.5 mL sample + 2 mL acetic acid-acetate buffer **b)** Addition of 20 µL 10^{-4} M metal ion **c)** Addition of 40 µL 10^{-4} M metal ion **d)** Addition of 60 µL 10^{-4} metal ion.

Table 1. The deposition conditions

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

Aerobic Training Protocol

Period: 6 week
Training frequency: 3 days a week
Training days: Monday, Wednesday, Friday
Training hours: 10–12 am
Training type: Continuous running at zero slopes
Training intensity: 60%–70%
Training period: 1 hour/session
Running distance: 5.6–6.8 km (changed according to the participant's heart rate)
Running speed: Determined in km / h in accordance to the target heart rate of each participants.
Equipment used: Treadmill, chronometer, telemetric heartbeat meter
Monitoring of heart beat rate: At 5^{th},15^{th},30^{th}, 45^{th} and 60^{th} minutes

Note: The heart beat rate of the participants during training was monitored with the use of digital sensor on the treadmill.

The intensity of the exercise was determined by the use of maximal heart beat rate protocol (maximal heart beat rate = 220-age; targeted heart beat rate = 0.75 × maximal heart beat rate) [7].

Nutrition Protocol

The participants were subjected to a predetermined, dietician controlled feeding protocol since they stay in the same student hall. The protocol started two days before the beginning of the training period. Utmost care was shown that the participants did not take any vitamin or mineral complex throughout the study.

Statistical analysis

Arithmetic means and standard deviations of the dependent variables were determined from the difference between the pre and final tests. The difference between the dependent variables were evaluated with t test with the use of SPSS software program at a significance level of $p<0.01$. The blood Zn and Cu levels were correlated with each other and max VO2 at a significance levels of ($p<0.01$) and ($p<0.05$) in the range of –1 and +1.

Results

When we compare pre and post training values of some physiological parameters we see that there are statistically significant difference at($P<0.01$) level between the body weights (76.42 ± 8.94 vs. 76.42 ± 8.94kg) and systolic blood pressure (124.17 ± 10.84 mmHg vs. 115.83 ± 5.15 mmHg). Although the diastolic blood pressure showed a decrease it was not statistically significant (75.00 ± 7.98 mmHg vs. 74.17 ± 5.15 mmHg). The pre and post training heart beat rate also showed a statistically significant decrease from 69.92 ± 1.98 to 68.17 ± 2.48 beat/min. The pre and post mean maxVO2 values on the other hand showed a significant increase from 45.51 ± 2.61 mL. kg/min to 49.12 ± 3.10 mL. kg/min ($P<0.01$).

The rest zinc levels before and after six week training period were 0.297 ± 0.099 ppm and 0.009 ± 0.005 ppm. The difference is statistically significant ($P<0.01$) (Table

Table 2. Statistics of the age and the height of the participants.

Variable	N	Arithmetic means (X)	SD	MinimalMaximal
Age (years)	12	21.67	0.89	20 –23
Height (cm)	12	180.08	6.07	170 – 189

The mean age and height of the participants were 21.67 ± 0.89 years and 180.08 ± 6.07 cm.

Table.3 Comparison of the pre and post 6-week training values of some parameters.

Parameter	N	Pre trainingvalue (X_1)	SD	Post trainingvalue (X_2)	SD	X_1–X_2	t.	p
Body weight (kg)	12	76.42 ± 8.94	2.58	74.50 ± 8.28	2.39	1.92	4.600*	0.001
Systolic blood pressure(mmHg)	12	124.17 ± 10.84	3.13	115.83 ± 5.15	1.49	8.34	3.458*	0.005
Diastolic blood pressure(mmHg)	12	75.00 ± 7.98	2.30	74.17 ± 5.15	1.49	0.83	0.561	0.586
Heart beat rate(beat/ min)	12	69.92 ± 1.98	0.57	68.17 ± 2.48	0.72	1.75	4.468*	0.001
MaxVO2(mL. kg/min)	12	45.51 ± 2.61	0.75	49.12 ± 3.10	0.89	3.61	**–5.678***	0.000

P<0.01*

Table 4. Comparison of the pre and post six week training mean trace metal levels in blood at rest

Variables		N	(X)	SD	X_1–X_2	t.	p
Zinc(ppm)	Pre six week training rest value	12	0.297± 0.099	0.029	0.288	**9.998***	0.000
	Post six week training rest value	12	0.009± 0.005	0.001			
Copper(ppm)	Pre six week training rest value	12	0.399± 0.146	0.042	0.157	**6.501***	0.000
	Post six week training rest value	12	0.242± 0.112	0.032			

P<0.01*

4). These values were found to be 0.399 ± 0.146 ppm and 0.242 ± 0.112 ppm for copper which also marked a statistically significant difference (P<0.01) (Table 4).

The pre and post six week training maximal loading blood zinc levels were found to be 0.294 ± 0.144 ppm and 0.019 ± 0.007 ppm. The difference is statistically significant (P<0.01) (Table 5). These values were 0.575 ± 0.258 and 0.363 ± 0.255 ppm for copper which also marked a statistically significant change (P<0.01).

Discussion

Normal resting state heart beat rate of a normal person is 70–80 beat/min. This value is 50 beat/min which decreases to 40–42 beat / min for high level marathon runners. Heart beat at rest (HBR) is closely related to the level of activity carried out [13, 20] . High stroke volume during sub maximal exercise indicates a good fitness level. It is known that endurance exercises decreases the heart beat rate and improves the stroke volume [13, 19] . Akova et al.[1] studied the effect of strenuous training protocol on 32 elite basketball and football players. There was also a control group of 12 sedentary people who were not subjected to any from of exercise. The heart beat rates at rest were found as follows: In basketball players 62 ± 10 beat/min, in football players 62 ± 6 beat/min in control group 78 ± 10 beat/min. This study also revealed a similar effect of long term exercise on the heart beat rate at rest and the average heart beat at rest was found to decrease from 69.92 ± 1.98 beat /min at the beginning to 68.17 ± 2.48 beat/min at the end.

Tulppo et al. [21] investigated the effect of aerobic training upon sedentary subjects and found that both the systolic and diastolic blood pressures showed an increase after the training. Penny et al.[16] reported that long term exercise resulted slight decrease in both systolic and diastolic blood pressures.

Aerobic power is described as the oxygen usage capacity (maxVO2). The average maxVO2 capacity is 4–5 L/min for normal people while it extends to 5–6 L/min for the sportsmen. This value is divided by body weight and given as mL/kg.min [19] . There are various field tests developed to measure this value [6, 19] . Darling et al. [4] applied continuous running program to 20 healthy university students and found an average maxVO2 value of 61.5 ± 7.7 mL/kg.min In this study an average maxVO2 value increased from 45.51 ± 2.61 mL/kg.min to 49.12 ± 3.10 mL/kg.min in complete accordance with the literature data

Zinc is an essential micronutrient for human body. There is no way to determine the slight zinc deficiency since there is no indication which shows this condition.

Table 5. Comparison of the pre and post six week training mean trace metal levels in blood after maximal aerobic loading

Variables		N	(X)	SH	X_1–X_2	t	p
Zinc(ppm)	Pre training maximal loading value	12	0.294 ± 0.144	0.041	0.275	**6.584***	0.000
	Post training maximal loading value	12	0.019 ± 0.007	0.002			
Copper(ppm)	Pre training maximal loading value	12	0,575 ± 0,258	0,075	0,212	**4,481***	0,001
	Post training maximal loading value	12	0,363 ± 0,255	0,073			

P<0.01*

Table 6: The correlation between the trace metal levels in blood and max VO2 values.

Variables	**Zinc**				**Copper**			
	Pre training rest value	Pre training max. loadvalue	Post training rest value	Post training max. loadvalue	Pre training rest value	Pre training max. loadvalue	Post training rest value	Posttraining max. loadvalue
Pre training MaxVo2	r = 0.03 p =0.925	r = 0.03 p =0.936	r = 0.54 p =0.071	r = 0.48 p =0.114	r = 0.16 p =0.612	**r = 0.75 p =0.005 (p<0.01)**	r = 0.44 p =0.153	**r = 0.58**
Post training MaxVo2	r = 0.02 p =0.957	r = −0.08 p =0.812	r = 0.57 p =0.06	**r = 0.76 p =0.004 (p<0.01)**	r = 0.20 p =0.530	**r = 0.71 p =0.010 (p<0.01)**	r = 0.40 p =0.202	**r = 0.68**

Acute zinc deficiency results in loss of weight, exhaustion, loss of endurance, osteoporosis and increase in viscosity of blood [14, 8].

Copper is also an essential micronutrient in human body which takes part more than 30 enzymes. However the studies showed no appreciable correlation between the copper level in blood and physical exercise. Although some workers claim that this ratio increases [5] as a result of physical activity there is no consensus here as well, as it is in zinc. Resina et al. [18] in their study they carried out in 1990 on the serum copper levels of 41 elite athletes and 24 people control groups found that the serum copper levels were lower in athletes. Marrella et al.[12] investigated the blood zinc and copper levels of 16 marathon runner before and after the race and found that there was a 29.3 % decrease in copper and 29.5 % increase in zinc levels. Lukaski et al.[10] in their study they carried out on pre and post racing season blood zinc and copper levels of 16 female and 13 male swimmers found that zinc levels increased and copper levels decreased at the end of the racing season (p>0.05). Ohno et al. [15] investigated the effect of strenuous exercise upon sedentary people and found the pre and post training blood copper values as 83.1 ± 5.1 and 79.9 ± 4.6µg 100mL-1. Brun et al.[2] studied serum zinc levels of 9 male and 11 female gymnasts and found that the levels of the girls were higher than the boys. Similarly Lukaski et al.[11] found that the blood zinc levels of female swimmers were lower and copper levels were higher than the males. They associated the difference between the performance of the males and females to the difference in blood zinc and copper levels. Cordova and Navas [3] investigated the effect of acute exercise upon the serum zinc levels of volleyball players and control group and found that the blood zinc levels showed an increase as a result exercise. Van Loan et al.[22] found that isokinetic extension resulted a decrease of 67 % in plasma zinc levels which cause an significant decrease in muscle strength and total work capacity. The blood values obtained in this study are as follows: For zinc; pre training period rest value before the aerobic loading 0.297 ± 0.099 ppm, pre training period value after maximal aerobic loading 0.294 ± 0.144 ppm, post training period rest value before the aerobic loading 0.009 ± 0.005 ppm and post training period value after maximal aerobic loading 0.019 ± 0.007 ppm. These values were found as 0.399 ± 0.146 ppm, 0.575 ± 0.258 ppm, 0.242 ± 0.112 and 0.363 ± 0.255 ppm for copper. These values are similar to some of the literature data.

In conclusion the level of trace of copper and zinc both as a result of maximal loading and a training period of six weeks showed statistically significant changes (p<0.01). When we compare the blood levels of pre and post six week training period we see a statistically significant decrease in both zinc and copper levels (p<0.01). The MaxVO2 values on the other hand have improved. It can be conveniently concluded that the consumption or discharge rates of both elements increase as a result of continuous exercise. Further studies are needed investigating the metal levels in urine and sweat to verify this fact.

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