Effect of Aerobic-anaerobic Exercises on the Concentrations of Some Blood Serum Elements

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Abstract

The aim of the study is to determine changes in several trace elements in erythrocytes such as cobalt, chromium, manganese, molybdenum and phosphorous in a chosen group within the students in Al-Esraa University College and the outcome of the study showed that a physical training everyday (may) motivate some variables in the concentration of some trace elements.

Keywords: Aerobic-anaerobic Exercise, Biochemical Test, Blood Serum and Trace Elements.

تأثير التمارين الهوائية–اللاهوائية على تركيز بعض العناصر

مكرم شكارة

كلية الاسراء الجامعة/قسم تقنيات المختبرات الطبية في مصل الدم

بغداد-العراق

الخلاصـة

الهدف من الدراسة هو تحديد التغيرات في عدد من العناصر النادرة مثل الكوبلت والكروم والمنغنيز والموليبديوم والفوسفور لدى عينة مختارة من طلبة كلية الأسراء الجامعة، واظهرت نتائج الدراسة ان التدريب البدني اليومي سيؤدي (ربما) الى اختلافات في تركيز بعض من هذه العناصر النزرة في مصل الدم.

الكلمات المفتاحية: التمارين الهوائية–اللاهوائية والفحوصات الحيوية ومصل الدم والعناصر النزرة

Introduction

Many researchers suggest that in general, many populations suffer from deficiencies in trace elements among their diets, which lead to the conclusion that many athletes (in these populations) may suffer from deficiencies in trace elements (Otag, *et al*, 2014; Heffernan, *et al*., 2019; Marino, *et al*., 2020).

There is not an easy method to measure the concentrations of trace elements in a human body. The most used method to determine the concentrations of trace elements in the serum or in the plasma, but a great fluctuation in assessments may be shown regardless of nutritious state (Khassaf, *et al*., 2001). Therefore, few studies tried to determine the effect of physical training on the concentrations of trace elements in erythrocytes comparing with studies that observed the effects of such training on macronutrients (Such as Calcium, Copper, Magnesium, Potassium and Iron) in erythrocytes (Oakes, *et al*., 2008).

Cobalt (Co) plays an important role as an antioxidant that inhibits or reduces the formation of reactive oxygen species. It, also, plays a crucial role in metabolic cycles during the production of hemoglobin and increasing the speed of erythropoiesis.

In addition to, cobalt has a role in dilating blood vessels inducing a hypotensive effect. It also regulates the pathway of methanol conversions (Berger, *et al*., 2002, Maynar, *et al*., 2018 a, b). Chromium (Cr) affects the lipid profile (By Decreasing Cholesterol and Triglycerides Levels) in a positive way since it involves in numerous enzymatic activities (Maynar, *et al*., 2018a). It was found recently that it accelerates the levels of glucose and encourages the cells to increase the number of insulin-receptors within it, which increases the activities of the muscles (Martin, *et al*., 2006). Manganese (Mn) is a critical micronutrient participates in Mn-superoxide dismutase (Mn-SOD) found in all human mitochondria. Mn-SOD is an antioxidant enzyme that catalyzes all superoxide radicals into free oxygen and hydrogen peroxide (H_2O_2). Human skeletal muscles have increased levels of SOD after vigorous exercise (Candas and Li, 2014). Molybdenum (Mo) participates in several redox processes, but contradictory results have been observed concerning its effect on physical activities. Some researchers could not observe any changes in the concentrations of molybdenum in plasma and urine after an extensive exercise or a marathon, while other researches show a contradiction results to this (Novotny and Peterson, 2018).

Both inorganic and organic phosphate salts are crucial in several metabolic cycles especially related to extensive sports exercises and performance. Actually, even with ambiguous results, some researchers believe phosphate compounds have serious impacts on aerobic as well as anaerobic producing-energy systems (Czuba, *et al*., 2009).

The deficiencies of phosphate compounds reduce contractions of the muscles of the heart, so reducing heart pulses.

In theory and with contradict experimental results; the presence of phosphate compounds will normalize the heart pulses during exercise. This means that the presence of phosphate salts will keep the pulses of the heart stable even during continuous endurance exercise (Czuba, *et al*., 2009; Mariño, *et al*., 2020).

Materials and Methods

Participations

Thirty-two sedentary participants aged 20.0 ±0.08 years were chosen as a control group. They have idle inactive lazy lifestyle. Twenty-five moderate-training participants aged

20.0± 0.11 years were chosen since they play different types of sports without systematic training. Twenty-one highly professional athletics ages 20.0± 0.84 years were chosen. They trained systematically and under a professional supervision.

All participants were voluntary non-smoking males with no health problems. None of them take (During the Study Period) any sort of supplements (Including Vitamins or Mineral Tablets) with complete understanding of the aims of this research. All were advised not to participate in any type of strenuous exercises a week before this study.

Anthropometric measurements

In Table (1), Oxygen Consumption (Vo) and Expiratory Volume (Ve) were higher in both the moderate-training group and athletics group comparing with the control group which indicate the high endurance intensity comparing of the individuals in both training groups.

Heart pulses and blood pressure (for each individual) were recorded during the rest period and after reaching the exhaustion point.

Participants in the control group show lower maximum heart rate comparing with the training groups, while normal resting heart rate was low in the training groups than in the control group.

The body weight (nearest 0.01 Kg) and height (up to 0.1 cm) of each were measured using a wall-mounted stadiometer and calibrated electronic digital scales (Seca 220. Hamburg, Germany) respectively.

Skinfold methods

Plicometer or skinfold caliber (GIMA-FIT Comp, Italy) was used to measure the subcutaneous adipose fat layer thickness (Under the Skin). Seven classical sites on the body were used. They are: Supraspinale, The anterior Thigh, Subscapular, the cheekbone, the manubrium of the breastbone (the sternum), 3-cm above the navel (the umbilicus) and Iliac Crest. Skinfold method is a sound firm method that assesses any changes in the body composition over a certain period of time.

It cannot be considered as an accurate method to measure the percentage of fat in the body. It must be said that experience is very important during using this technique.

The sum of seven skinfolds (∑6) (Mentioned Above) was estimated and represented in Table (1).

Wingate accumulative tests

This anaerobic test assesses strength, agility and speed was used as in the previous paper (Yunis, *et al.*, 2018) to evaluate the performance variables, using a cycle ergometer with an analyzer and a pulsometer (Medical Expo, UK).

In case of all three groups, the exercise protocol consists of 2 min at rest, 20 min build-up ending with 6 min at 50 watts, and then opening at 70 watts and increasing the intensity by 15 watts every 5 min until exhaustion.

All tests were done at room temperature (24–27 °C). Heart rate and blood pressure (For Each Individual) were measured during the rest period and after the end of the tests.

Evaluation of daily Nutrition

All participants, on each day of the experiment, will report the type and quantity (in grams as far as possible) of every food they ate in the previous 24 h. The nutritional composition of their diets was evaluated using food composition tables as indicated in (Reilly, 2004).

Sample collection

Blood samples

5 mL of venous blood were collected from all participants before and after any test.

Table (1) The Features of the Groups Involved in this Study

	Sedentary Group (N = 32) (CG) (Control Group)	Moderate - Training Group (n = 25) (MTG)	Athletics Group (n = 21) (AG)
Height (m)	1.77 ± 0.05	1.73 ± 0.07	1.75 ± 0.18
Weight (Kg)	71.00 ± 3.11†*	67.34 ± 2.03	64.12 ± 3.16
Age (Years)	20 ± 0.08	20.00 ± 0.11	20.00 ± 0.84
Training Period (Years)	None	6.00 ± 0.15	8.00 ± 3.05
Σ6 Skinfolds (mm)	115.34 ± 25.36	92.65 ± 1.22+	74.29 ± 2.43†*
$VO_{2Máx}$ ($ml{\cdot}min^{-1}{\cdot}Kg^{-1}$)	29.22 ± 1.14	41.81 ± 3.66++	58.11 ± 1.11††**
V_{Emax}	83.16 ± 7.22	110.32 ± 1.33++	142.54 ± 2.44††**
HR_{rest} ($pulses{\cdot}min^{-1}$)	68.33 ± 2.07	64.90 ± 3.85++	60.15 ± 1.43††**
HR_{max} ($pulses{\cdot}min^{-1}$)	154.32 ± 1.03	146.96 ± 2.31+	140.71 ± 1.09†

Anova and Bonferroni tests
† Differences between the AG and CG (†$p < 0.05$, ††$p < 0.01$, †††$p < 0.001$)
+ Differences between the MTG and CG (+$p < 0.05$, ++$p < 0.01$, +++$p < 0.001$)
* Differences between the AG and MTG (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

All participants practice 8 h-fasting period before the test.
Each sample was centrifuged at 3000 x g for 10 min and the participated pellets (erythrocytes) were washed with 0.8% sodium chloride (NaCl) three times. The pellets erythrocytes were moving into Eppendorf tubes and stored at − 80 °C until biochemical analysis.

Determination of Hemoglobin and Hematocrit

Blood (In a Glass Capillaries Containing Heparin) was centrifuged inside a Microcen microfuge (Ascorde, England) to obtain hematocrits.

Hemoglobin (Hb) was determined using a Hb analyzer (Hillcrot, Sweden).

Erythrocyte element determination

Sample preparation

A standard mineral solution (Cobalt, Chromium, Manganese, Molybdenum, Phosphorous) of 1,000 ppm was dissolved using a proper weight for each mineral oxide (Such as $CoSO_4$, $MnSO_4$, etc.) in 1 liter of distilled water. Standards (of 5 to 300 ug) were prepared by dilution with distilled water. Heart pulses and the pressure of the blood (of each participant) were measured using automatic wrist digital blood pressure (Shantez, Germany) respectively before and after each test.

Mineral Tests

2 ml of (Any Trace Element) standards was mixed with 2 ml 4N HCl, and 2 ml 0-1% ammonium pyrrolidine dithiocarbamate (APD) was added to the mixture after it was left to stand for 10 minutes at room temperature.

After careful mixing, 2 ml n-butyl acetate was added and the mixture shakes for 10 minutes and centrifuge for 5 minutes, and then pipette off the top n-butyl acetate layer with a glass Pasteur pipette. 2 mL of deionized water was added as a blank. The absorption was read at a suitable wavelength (According to Each Trace Element) using the BWB

XP flame atomic photometer (BWB Technologies.

Statistical Analysis

In the text, data were presented as mean ± standard deviation (S.D.). Any comparing two groups' data was analyzed by unpaired t-tests. The curves in the trace element experiments and metabolic syndrome models were analyzed by two-way ANOVA, followed by Bonferroni's post hoc test. Statistical significance was defined as $p < 0.05$.

To ascertain a relationship between the physical training and the changes in erythrocytes in the concentrations of the trace elements, a Pearson correlation study was carried out. A significant difference was considered when $p < 0.05$.

Results

The total weight and body fat percentage decreased significantly in moderate training group and athletics group comparing with sedentary control group while and maximal VO2 and VE were increased in both training groups comparing with the control group as an indication of the effects of continuous exercises as illustrated in Table (1). The basal (Resting) HR was higher in the control group comparing with both training groups, while maximal HR was higher in the training groups comparing with the control group which is expected as shown in Table (1). The results showed no significant differences (Except Chromium) between the three groups considering the taking of daily trace elements and this represented in Table (2) or in the concentrations of hemoglobin and hematocrit as illustrated in Table (3). Hemoglobin was shown as the main protein present in erythrocytes (Table 3).

The concentration of each trace element (Under Study) inside erythrocytes was assessed and expressed using (µg/g Hb). The results of the comparisons between the three groups are shown in Table (4). The correlation of the concentrations of the trace elements Co, Cr, Mn, Mo, and P with the level of training is shown in Table (5). The results were expressed with a correlation coefficient (r) and a significance level (p).

Table (2) The Daily Consuming of Co, Cr, Mn, Mo, and P in the Three Groups

	Control Group (CG) (n = 32)	Moderate - Training Group (MTG) (n=25)	Athletics Group (AG) (n = 21)
Co (3–100 µg/d)	75.30 ± 12.72	78.03 ± 8.22	77.14 ± 2.34
Cr (20–300 µg/d)	163.3 ± 22.5	178.5 ± 23.1	188.3 ± 13.1
Mn (3–100 µg/d)	245.2 ± 2.7	251.1 ± 5.3	255.4 ± 1.5
Mo (50–500 µg/d)	241.1 ± 14.3	241.0 ± 16.4	242.21 ± 14.1
P (500–3000 µg/d)	121.8 ± 1.15	120.4 ± 1.14	123.31 ± 1.05

Table (3) Concentration of Hemoglobin and Hematocrit in the Three Groups

	Control Group (CG) (n = 32)	Moderate - Training Group (MTG) (n=25)	Athletics Group (AG) (n = 21)
Hemoglobin (g/dL)	15.11 ± 1.21	16.21 ± 1.54	16.32 ± 1.76
Hematocrit (%)	40.11 ± 1.01	40.75 ± 0.92	42.12 ± 1.07

Table (4) The Concentrations of Co, Cr, Mn, Mo and P in the Three Groups After Training Periods

	Sedentary Group (N = 32) (CG) (Control Group)	Moderate - Training Group (n = 25) (MTG)	Athletics Group (n = 21) (AG)
Co (μg/gHb)	0.29± 0.09	0.24 ± 0.12+	0.11 ± 0.16†*
Cr (μg/gHb)	0.40 ± 2.03	0. 34 ± 1.36+	0. 28 ± 2.22†††
Mn (μg/ gHb)	2.63 ± 0.13	1.89 ± 0.08++	1.15 ± 0.22††
Mo (μg/ gHb)	0.28 ± 0.04	0.16 ± 0.16+	0.09 ± 0.02†*
P (μg/ gHb)	2.45 ± 0.40	1.75 ± 1.01+	1.03 ± 1.25†

ANOVA and Bonferroni tests
† Differences between the AG and CG (†p < 0.05, ††p < 0.01, †††p < 0.001)
+ Differences between the MTG and CG (+p < 0.05, ++p < 0.01, +++p < 0.001)
* Differences between the AG and MTG (*p < 0.05, **p < 0.01, ***p < 0.001)

Table (5) The Correlations between the Trace Elements and the Level of Training Represented by the r.

Element	Co	Cr	Mn	Mo	P
Training Status	− 0.372, p = 0.113	− 0.182, p = 0.163	− 0.533, p = 0.102	− 0.072, p = 0.091	− 0.262, p = 0.011

Discussion

Heart rate can be used as index showing the physical stress during exercises. It must be noted that even the athletics students are not a professional athlete which can explain the lower maximal HR comparing with researchers used true professional athletes. Previous researchers assess the effects of cobalt (Co) show contradiction results. In one study, the concentration of cobalt remains stable without any significant changes in marathon running women, while other researchers show that a small but significant increase in the concentration of cobalt during marathon running (Apple, *et al*, 2002; Berger, *et al*, 2002; Day and Thompson, 2010).

Maynar, *et al*., (2018b) found that the concentration of cobalt remains stable in both control and athletes group participating in aerobic and anaerobic modalities but found that cobalt concentration in the athletic group rises significantly comparing to the control group in one research concerning the soccer players (Aerobic-anaerobic).

During our research, higher concentration of cobalt was found in the control group and decreased in athletic group giving a high a high negative correlation ($r = -0.790$, $p < 0.001$) with the degree of training. This may be due to the activity of Co-Zn-SOD enzyme in erythrocytes which increased rapidly with severe training, while becomes inactive in sedentary participants (Tara, *et al*., 2012, Kenneth, *et al*., 2012).

Most cobalt compounds act as antioxidants that protect the muscles of the body from free radicals formed during natural actions of the body. These antioxidant properties play an essential role in the defense against free radicals, which occurs largely in situations of trauma and overexertion as well as during strenuous exercises. Chromium is one of the trace elements that control the level of insulin, and thus influence, in a way, the metabolism of carbohydrates, lipids and proteins (Martin, *et al*., 2006).

The hypothesis that chromium is deficient from general population stated in some research papers were not founded recently (Berger *et al*., 2002). Contrarily, others stated that that the concentration of chromium in the serum in different sports modalities was significantly higher in athletes than in a control group (Lee, *et al*., 2017; Maynar, *et al*., 2018 a, b). In our study, the concentration of chromium was declined with the extensive of training, even though the number of erythrocytes increases with the severe level of training. It is probably, as stated by Lukasiki (2000), excess chromium produced during exercises will move to the kidneys and removed through urination.

In mammalian cells, manganese (Mn) is present in the structures of many enzymes such as manganese superoxide dismutase (Mn-SOD), which is an antioxidant enzyme that present in mitochondria and responsible for the detoxification of free radical superoxide, one of the by-products during aerobic and anaerobic respiration (Hu, *et al*., 2005, Bugckent, *et al*., 2019). Previous studies indicated an increase in the activity of Mn-SOD during training, which explain the changes in the concentration of manganese since the level of the free radicals will increases throughout the training (Miriyalaa, *et al*., 2012, Candas and Li, 2014).

Manganese played an important role in gluconeogenesis and its relationship to the aerobic training and the formation of urea (Bugckent, *et al*., 2019).

According to Maynar, *et al*., (2018b), the concentration of manganese is higher in people exercised daily comparing to sedentary idle people, but the concentration of this element will be much lower comparing to the control group in professional athletes.

In our study, our results agreed with that, that is, the concentrations of manganese in erythrocytes are higher in control group, but decreased with the level of training, reaching its lowest concentration in the athletic group.

The absence of mitochondria from erythrocytes means the absence of Mn-SOD in them, so the increase of manganese trace element or its increase may be due to the presence of some type of reservoir in the body which can manifest this element. This needs to be investigated further (Hu, *et al*., 2005).

In this study, Molybdenum concentration inside erythrocytes was lower in the two training groups comparing with the sedentary control group. This may lead to the production of uric acid which plays as an antioxidant that will prevent the superoxide anions from damaging the muscles of the trainees during intensive exercises. Uric acid was formed as a by-product of a purine nucleotide cycle in which molybdenum involves, so, it seems to be a kind of a correlation between molybdenum and an extensive training period (Maynar, *et al*., 2018 a).

Phosphate compounds effect human metabolism, especially during sport performance. This may (or may not) influence aerobic and anaerobic metabolism systems. Phosphate loading seems to improve aerobic metabolism which may be caused by the phosphates that participate in the formations of 2,3-diphosphoglycerate (2,3-DPG), which facilitates the release of oxygen (In Red Blood Cells) to the tissues. There are many hypotheses, but unfortunately, the results in this area are ambiguous (Baker, *et al*., 2010, Buck, *et a*l., 2014).

In our study, the concentration of phosphate compounds (Inside Erythrocytes) was higher in the control group comparing with the other two groups.

The deficiencies of phosphate, theoretically, affected the contractile properties of the muscles of the heart muscle, so intake of phosphate salts will increase the resting and maximal heart. Several researchers supported this hypothesis (Czuba, *et al*., 2009, Baker, *et al*., 2010). In general, phosphate salts (though ambiguous results) are taken by athletics regularly particularly during races that take 2-3 weeks (Such as Cycling Races). Athletes believe (Seriously) that this will affect their competitions through different races. It was suggested that since the concentrations of trace elements in erythrocytes are not affected by short time but may depend on the type and degree of training (Lee, *et al*., 2017).

The role of antioxidant compounds that formed by trace elements and defend the body against free radicals formed during periods of stress, exhaustion and overexertion need to be studied thoroughly.

Conclusions

In conclusion, our study showed that all training groups (and depending on the severity of the training) have deficiencies in cobalt, chromium, molybdenum, manganese, and phosphate, while sedentary participants do not suffer from such deficiency. So, the evaluation of erythrocytes must be carried out in all athletes in our belief before, during and after training.

The area of this research must be extended to cover all trace elements in the body to include them in the diet during training. Previous studies showed low concentration of trace elements in the athletes' serum (De Lisio*, et al.*, 2011; Marbauri, 2013; Diaba-Nuhoho, *et al*., 2018).

All these studies suggested the need of such elements in the diet, but, in our opinion, a low value of any trace element is not a signal of deficits, if the concentrations of those trace elements are higher inside the cells which can be consider and a storage room. More studies are in need to determine the concentrations of trace elements inside erythrocytes as well as in the serum before ascertain and suggest possible and order any supplementation to the diet of athletes.

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