



Original Research

Effects of morning and nocturnal soccer matches on levels of some trace elements in young trained males

Sermin Algul^{1*}, Aydin Sukru Bengu², Saltuk Bugra Baltaci³, Oguz Ozcelik⁴

¹ Department of Physiology, Faculty of Medicine, Van Yuzuncu Yil University, Van, Turkey

² Department of Medical Services and Techniques, Vocational School of Health Services, Bingol University, Bingol, Turkey

³ Department of Physiology, Faculty of Medicine, Selcuk University, Konya, Turkey

⁴ Department of Physiology, Faculty of Medicine, Firat University, Elazig, Turkey

Correspondence to: serminalgul@hotmail.com, serminalgul@yyu.edu.tr

Received August 6, 2018; Accepted February 14, 2019; Published February 28, 2019

Doi: <http://dx.doi.org/10.14715/cmb/2019.65.2.6>

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Abstract: The aim of this study was to comparatively evaluate effect of morning and nocturnal soccer matches induced metabolic stress on plasma levels of iron (Fe), copper (Cu) and zinc (Zn). Twenty male footballers performed two soccer matches in morning and at night on different days. Blood samples were taken before and after match. The levels of Fe, Zn and Cu were measured through an atomic absorption spectrophotometry. Metabolic stress was evaluated by altered malondialdehyde (MDA) levels that measured using High Performance Liquid Chromatography. In morning and at nocturnal soccer matches, levels of MDA (36% and 27%), Fe (37.4% and 38.9%) and Cu (34.8% and 26.8%) were all increased in all subjects, respectively. However, Zn level decreased -4.5 % in morning (n=10 subjects) and -9.4% at nocturnal (n=12 subjects) soccer matches. In addition, Cu/Zn ratio increased significantly 46.6% in morning and 36.6% at nocturnal soccer matches. Soccer match has significant effects on levels of MDA, Fe and Cu but not Zn levels. The results of this study showed that morning soccer match significantly alters levels of MDA and Cu and Cu/Zn ratio compared to nocturnal soccer match.

Key words: Exercise time; Aerobic exercise; Trace elements; Metabolism; Oxidative stress.

Introduction

The beneficial roles of trace element in many physiological metabolic functions of biological systems have been shown (1). The regulation of trace elements in blood and tissues should be kept within optimal limits to support metabolic functions of the body systems at rest at also during exercise (2). Importantly; physical exercise may cause redistribution of some trace elements between body stores, blood and tissues (2, 3).

Exercise is an effective stimulatory factor on energy metabolic systems (4). In addition acute exercise may cause a significant increase in levels of oxidative stress in body (5, 6). Therefore, evaluation of trace element variations in response to the exercise induced increased metabolic activity is important point that needs to be clarify.

Iron (Fe), copper (Cu) and zinc (Zn) are essential trace elements responsible for the function of several enzymes and proteins that have important roles in body metabolic systems regulation. In addition, an imbalance in Fe, Zn and Cu regulation results serious consequences with regarding cell function and may lead neurodegenerative disorders and increased oxidative stress (7, 8). Cu and Zn have protective effects against increased free radical species (9, 10). Fe, in the form of haemoglobin, is an essential element that has a crucial role in the oxygen transportation from lung to tissues, and related to the exercise performance and metabolism

(9, 10). A variety of studies with different experimental design, including aerobic, anaerobic or endurance exercise have been performed to obtain some information about the relationships between trace elements of Fe, Cu and Zn and exercise. Many studies showed that various intensity of exercise has significant effects on trace element metabolism (8, 11-14). In contrast, unchanged trace elements levels after acute exercise has also been reported (15).

However, there is no satisfactory information concerning Fe, Cu and Zn in response to soccer match induced metabolic stress (16). In addition, little is known about the beneficial or detrimental effects of soccer matches induced metabolic stress (include aerobic and anaerobic exercise performances) on trace elements with regarding time of morning and at night.

The purpose of this study was to evaluate how soccer matches induced metabolic stress affect plasma levels of iron (Fe), copper (Cu), zinc (Zn) and cooper to zinc ratio (Cu/Zn) with regarding time of morning and night in trained male subjects.

Materials and Methods

Subjects

This study was conducted with twenty healthy male subjects who were actively engaged in soccer matches (data collected from the goalkeepers of each team were not included in the study). Mean age of the subjects was

20.3 ± 0.5 years, mean height was 175.7 ± 10 cm and mean body weight was 61.7 ± 7.6 kg. This study protocol was approved by Firat University Non-Interventional Ethics Committee. Before starting the study, the informed consent form was obtained from the subjects who participated to the study. The protocol of this study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Training healthy male subjects aged 18-25 years were included in this study. Criteria sought in training subjects is to perform sport highly as a licensed amateur or professional (at least 3 years since they have performed sports and participated in weekly regular training). Subjects who have acute (influenza infection, sore throat, inflammation in the muscles etc.) or chronic illnesses (diabetes, obesity, allergy, myocardial insufficiency) or metabolic, respiratory, cardiac and skeletal muscle system disorders that may affect the results of the study were not included in the study. In addition, alcohol, cigarettes, and regular drug users were also not included in the study.

Exercise protocols

The soccer match performed in the present study was a friendly game played by local amateur licensed players. The subjects performed two soccer games on different days, one in the morning (between 08:00 to 10:00) and one at night (between 21:00 and 23:00) for entertainment purposes. The soccer match was performed on an average of 2 x 45 minutes (with 15 minutes rest). Subjects had a break at least three days between soccer matches. During study period, they were asked to keep their eating habits unchanged and stay away from energy drinks, vitamins, and coffee or performance influencer substances.

Blood collection

Venous blood samples were taken from vein in to EDTA-aprotinin containing tubes shortly before match and immediately after match. All blood samples were centrifuged at 4500 rpm for 5 min at + 4 °C and stored at -80 °C until plasma analysed.

MDA analyses

The subjects' metabolic stress during soccer games were evaluated using plasma malondialdehyde level (MDA) (17). The samples were analysed for MDA in a double-blind condition. Serum MDA levels were determined by High Performance Liquid Chromatography (HPLC) methods using commercial kit (Immu Chrom GmbH Tiergartenstr. 7 D 64646 Heppenheim IC 1900). The intra and inter-assay of variation and sensitivity for MDA were 9% (0.86 µmol/L) - 6.4% (2.55 µmol/L), 10.9% (0.89 µmol/L) - 7.5% (2.5 µmol/L), respectively. MDA measurement using HPLC is the most favourable methods for accurate detection of MDA in sports and exercise area due to its sensitivity and accuracy (18).

Plasma copper, iron, zinc analyses

Atomic absorption spectrophotometer is used to determine the amount of metal elements. The principle of this method is based on the excitation of the free atoms of the element by absorbing the rays in the ultraviolet or

visible region. This process is accomplished by incandescing the container, for example by putting into the carbon sample container, that containing the element as a compound with an electric arc.

Determination of Fe, Cu, Zn levels in the plasma was executed in Atomic Absorption Spectrophotometer (Perkin Elmer AAS 800, USA). Measurements were made twice for each sample with light at wavelengths of 248.3 nm, 324.8 nm and 213.9 nm, respectively, for the elements through the flaming atomisation technique. The levels of mentioned elements were determined as ppm.

Statistical analysis

Data are expressed as mean (± S.D.). The Paired-t test, which is a parametric comparison, was used to analyse the significance of within-training group comparisons of data. A value of $p < 0.05$ was accepted as statistically significant.

Results

MDA levels

The soccer match induced increase in oxidative stress level defined by enhanced MDA level. MDA levels increased in all subjects when compared to pre-match values 36% in morning (from 0.717 ± 0.04 µmol/L to 0.977 ± 0.04 µmol/L $p < 0.001$) and 27% at night (from 0.776 ± 0.03 µmol/L to 0.991 ± 0.04 µmol/L $p < 0.001$) (Table 1).

Fe levels

Plasma Fe levels showed a progressive and significant increase from baseline levels until end of soccer match in morning (5.305 ± 0.02 ppm and 7.292 ± 0.02 ppm) ($p < 0.001$) and at night (5.223 ± 0.01 ppm and 7.256 ± 0.01 ppm) ($p < 0.001$) (Table 1). The increase of Fe levels was found to be 37.4% in morning and 38.9% at nocturnal soccer match (Table 1). There was no statistical difference in basal Fe levels in both soccer matches.

Zn levels

Plasma Zn level showed variations during soccer matches among the subjects in the morning (n=10 increases n=10 decreases) and at night (n=8 increases n=12 decreases) (Figure 1). The Zn levels were decreased from 1.125 ± 0.06 ppm to 1.074 ± 0.05 ppm

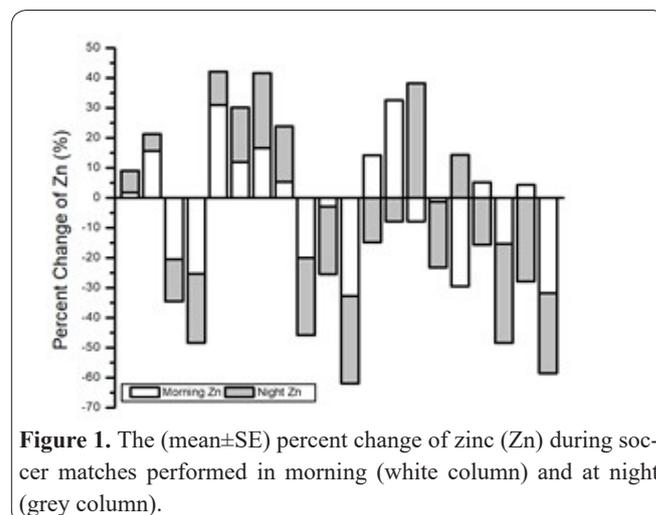


Figure 1. The (mean±SE) percent change of zinc (Zn) during soccer matches performed in morning (white column) and at night (grey column).

Table 1: The mean (\pm SE) values of malondialdehyde (MDA), iron (Fe), copper (Cu), zinc (Zn) and zinc to copper ratio (Cu/Zn) before and after soccer match performed in morning and at night.

Morning	Night			P	Night		
	Basal	End match	P		Basal	End match	P
MDA (μ mol/L)	0.717 \pm 0.04	0.977 \pm 0.04	P<0.001	0.776 \pm 0.03	0.991 \pm 0.04	P<0.001	
Fe (ppm)	5.305 \pm 0.02	7.292 \pm 0.02	P<0.001	5.223 \pm 0.01	7.256 \pm 0.01	P<0.001	
Cu (ppm)	0.129 \pm 0.01	0.174 \pm 0.01	P<0.001	0.302 \pm 0.01	0.383 \pm 0.01	P<0.001	
Zn (ppm)	1.125 \pm 0.06	1.074 \pm 0.05	P=0.1	1.181 \pm 0.05	1.069 \pm 0.03	P=0.1	
Cu/Zn ratio	0.120 \pm 0.01	0.176 \pm 0.01	P<0.001	0.270 \pm 0.02	0.369 \pm 0.02	P<0.001	

in the morning (-4.5%) and from 1.181 \pm 0.05 ppm to 1.069 \pm 0.03 ppm at nocturnal (-9.4%) matches (Table 1). However, these decreases were not statistically different from their basal levels (Table 1).

Cu levels

Plasma Cu levels showed a progressive and significant increase from baseline levels until end of soccer game in morning (0.129 \pm 0.01 ppm and 0.174 \pm 0.01 ppm) (p <0.0001) and at night (0.302 \pm 0.01 ppm and 0.383 \pm 0.01 ppm) (p <0.001) (Table 1). The increases in Cu levels were found to be 34.8% in morning and 26.8% at nocturnal soccer matches. In contrast to the Fe and Zn, there was statistically significant difference in basal Cu levels between morning and nocturnal soccer matches (p <0.001) (Table 1).

Cu/Zn ratio

The Cu/Zn ratio significantly increased from 0.120 \pm 0.01 to 0.176 \pm 0.01 (P <0.001) (46.6%) in morning and also from 0.270 \pm 0.02 to 0.369 \pm 0.02 (P <0.001) (36.6%) at nocturnal soccer matches (Table 1). There was significant differences in basal Zn/Cu levels in both soccer matches (P <0.001) (Figure 2).

Discussion

The purpose of this study was to investigate the effects of soccer match performed in morning and at night on blood Fe, Zn, Cu and Zn/Cu ratio in trained young male subjects. A standard (2x45 min) soccer match effort implies several acute physiological stress in body systems, including increased cardiac output, blood flow, metabolic activity and muscle contractions.

In literature, experimental evidence indicates that acute exercise induced metabolic stress elevates blood MDA levels (5, 18). The findings of present study show that soccer match performed in morning and at night caused significant increases in MDA levels comparison to pre-exercise values (Table 1). However, increased MDA level was significantly higher in morning compared to night soccer match (Table 1). The diurnal effects of high cortisol levels in morning could be the reason for this difference in MDA increase levels (19).

The soccer match induced metabolic stress showed significant influence on levels of plasma trace elements, especially Fe, Zn, Cu and Zn/Cu ratio.

In this study, there was systematic increases plasma Fe levels in all subjects in both soccer matches compared to their pre-match values (Table 1). In literature, it has been reported that rats subjected to acute swimming exercise elevated serum iron levels compared to the control which is consistent obtained in present study

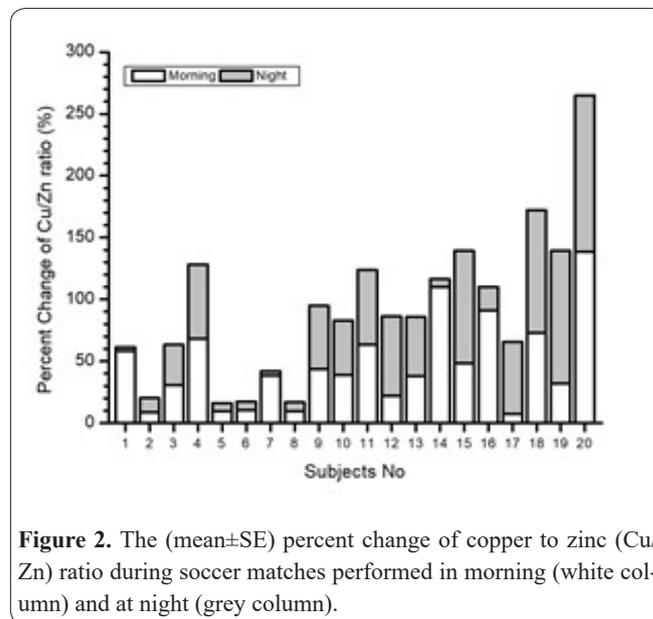


Figure 2. The (mean \pm SE) percent change of copper to zinc (Cu/Zn) ratio during soccer matches performed in morning (white column) and at night (grey column).

(11). This finding could be related with tissue damaged induced iron release to extracellular fluids (20). Fe is an important trace element that has a crucial role in O₂ transport to support increased metabolic demands of exercising muscle. It is shown that iron may cause improved physical capacity during exercise (21). The increased percentage of Fe level was significantly higher in nocturnal soccer match compared to morning match (Figure 1). This could be further increase in Fe levels in night soccer match could be related with the circadian effects (22). The increased Fe levels in nocturnal exhaustive treadmill exercise have also been shown (23). However, in contrast to our results a transient decreases in iron levels 2 hours after soccer matches has been reported (24).

The evaluation of the relationships between soccer matches performed morning and at night on Cu metabolism showed systematic increases in all subjects. This is also support that acute aerobic swimming exercise induced increase in serum Cu levels in rats (2). Our study showed a higher percent increase in plasma Cu levels in trained subjects especially after morning soccer match compared to those performed at night (Table 1). It is known that Cu is a critical nutrition involved in some part of energy metabolism, myoglobin and some peptides hormones (9). Metabolic stress seems to be important factor for Cu levels. Increased Cu during soccer matches may be results of increased oxidative stress as determined increased MDA levels (25). It has been shown that serum Cu concentration was significantly and directly related to lipid peroxidation (26). In addition, Cu causes an acceleration of lipid peroxidation in human erythrocytes (27). A circadian effect could be

reason for the higher basal Cu levels in night soccer match compared to morning match (28).

In the present study, percentage increase of Cu levels (34.8% in morning and 26.8% at night) was not statistically different that percentage increase of MDA levels (36% in morning and 27% night) (Table 1). Decrease in Cu levels after acute maximal aerobic exercise (29, 30) and increased Cu levels after anaerobic exercise compared to aerobic exercise have been reported (31). In addition, increase in Cu levels after soccer match also support the suggestion of hemoconcentration mechanism that results natural consequence of exercise (32).

Numerous studies have focused on the relationship between physical exercise and Zn levels (33-35). It is logical to expect that exercise induced soft tissue injury may effects plasma Zn levels. The systemic availability of Zn in cells is highly influenced by the balance of anabolic and catabolic processes regulating the renewal of soft and skeletal tissues (9, 36). However, the data in literature on changes in Zn levels in response to the exercise are contradictory. In the present study, in contrast to systematic increases in Fe and Cu levels, reduced Zn levels were found for some but not all subjects and no significant differences were found in plasma Zn levels from baseline to end of match. This finding is in agreement with the results of several authors who observed no differences (15, 35, 37). The small but non-significant decrease in Zn levels after both soccer matches could be related with shift of Zn from blood to cell (3). Interestingly, there is no significant effect of soccer match on plasma Zn status in morning and at night. It has been shown that Zn ions are delivered into organ and tissues by specific transporters under the condition of increased oxidative stress (38).

However, increased Zn levels in sedentary subjects and decrease in Zn levels in trained subjects has been reported (33). In addition, endurance runners have been shown significantly lower serum Zn levels than untrained subjects (39). However, increase in Zn levels following exercise has been reported (30, 40).

Increased metabolic stress in muscle indicated by a rise in MDA levels during soccer matches play a significant role in raising levels of Cu/Zn ratio (Figure 2) (26). The increased Cu/Zn ratio has been shown to have important inflammatory and nutritional biomarker in human health (41).

As a conclusion, soccer match performed in morning and at night caused significant increases in levels of MDA, Fe, Cu and Cu/Zn ratio; small but non-significant decreases in Zn levels. MDA and Cu levels were found to be significantly lower in night match compared to morning match. However, the relationships between plasma concentration of these trace elements and their tissue concentration are still unclear and need to be clarifying.

Acknowledgements

N/A

Conflict of interest statement

The authors declared no conflict of interests regarding the publication of this manuscript.

Authors' contributions

Each author have participated sufficiently in the work to take public responsibility for appropriate portions of the content. All authors have read and approved the final version of the manuscript and agree with the order of presentation of the authors.

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