

Response of Some Trace Elements to Soccer Match Performed Different Times of Day in Young Sedentary Male

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Abstract. *Objective:* We aimed to examine effects of soccer match induced metabolic stress on levels of some trace elements. *Materials and Methods:* Fourteen sedentary male subjects randomly performed three indoor soccer matches (2 x 30 minute) in morning, afternoon and at night on different days. Venous blood samples were taken before and after match. Plasma malondialdehyde levels measured by High Performance Liquid Chromatography. Total antioxidant capacity and total oxidant capacity were estimated using a commercially available enzyme-linked immunosorbent assay kit. Plasma iron, copper and zinc levels was measured using an Atomic Absorption Spectrophotometer. *Results:* Significant increases were found in iron, malondialdehyde and total oxidant capacity in all matches ($p < 0.0001$). Zinc decreased ($p < 0.0001$) in morning but increases at night soccer a match ($p < 0.0001$) which is opposite to copper. *Conclusions:* There was no significant correlation between increased stress parameters and trace element levels. However, time of soccer match causes opposite influence on levels of zinc and copper.

Key words: Exercise, Malondialdehyde, Total oxidant capacity, Total antioxidant capacity, Zinc, Iron, Copper

Introduction

Regular exercise training or increased physical activity has favourable effects on many established risk factors for metabolic or cardiovascular disease (1,2). However, exercise induced metabolic stress causes many challenge in body homeostasis and may have effects on oxidative balance and trace elements (3,4). Many sedentary people are involved in various type of physical activity to improve their health. Indoor soccer game is widely preferred leisure time physical activity among sedentary male subjects, during different times of the day. Soccer game includes wide exercise intensity option from lower to higher intensity (5).

It is known that soccer game increases oxidative stress and metabolic damage and muscular strain in trained subjects (6). Some trace elements, including iron (Fe), Zinc (Zn) and copper (Cu) have significant roles in enzyme regulation of metabolic activity and protective effects on increased oxidative stress (6-10). The trace element distribution between tissue and blood should be regulated within their optimal levels (11). Trace elements response to the different type of exercise has been studied in various subjects groups and results are varied among the studies. However, comparison of exercise induced stress on trace element in various times of day in sedentary subjects has not been well investigated. This is because body metabolic

system could be affected by the time of day with regarding circadian clock (12).

We investigated the effects of acute metabolic stress induced by indoor soccer games performed in morning, afternoon and at night on oxidative stress levels in sedentary male and its relationships with the levels of some trace elements of Fe, Zn and Cu.

Materials and methods

Participants

A total of fourteen sedentary male volunteered to participate in this study. Mean (\pm SD) values for the age, height and body weight were 21.5 ± 1.1 yr., 176.2 ± 5 cm and 63.4 ± 9 kg respectively. All subjects were free of cardiovascular, neuromuscular, metabolic or any systemic diseases and taking no medications or nutritional supplements. In addition they were also instructed to refrain from caffeine, smoker and alcohol. The subjects were informed the potential benefits and risks associated with the study. Each subject gave signed written informed consent before participating in the study. The study procedures were approved by the Medical Ethic Committee of the Firat University in accordance with the Declaration of Helsinki. The body composition of the subjects was examined using bioelectric impedance analysis method in the morning on the day of first soccer match.

Exercise Protocol

The indoor soccer matches performed in 50 m length and 30 m wide field with covered synthetic carpet for one hour (30 min x 2) without rest. They played three soccer matches on different days: one in the morning between 8:00 to 10:00 am, one in the afternoon between 14:00 to 16:00 and one is at the night between 22:00 to 24:00. The all soccer matches were played randomly and as a free time leisure activity. There was 3 days between each soccer matches. During study period, they were asked to avoid energy contains drinks, vitamin supplements, tea coffee or any drugs that may effects performance and changing their eating habits.

Blood Collections

A five ml venous blood sample was taken at rest and at the end of the match in to EDTA- aprotinin containing tube. The samples were centrifuged at 4C for 5 min 4500 rpm to separate the plasma. The plasma was aliquoted in to Eppendorf tubes and stored at -80C until further analysis.

Metabolic and Oxidative Stress Parameters:

MDA Analyses

Plasma malondialdehyde levels (MDA) were used to analyse metabolic stress levels of the subjects before and after soccer matches (13). MDA levels were measured by High Performance Liquid Chromatography (HPLC) which is favourable methods for accurate and sensitive detection of MDA, using commercial kit (Immu Chrom GmbH Tiergartenstr. 7 D 64646 Hepenheim IC 1900). The intra and inter-assay of variation and sensitivity for MDA were 9% ($0.86 \mu\text{mol/L}$) - 6.4% ($2.55 \mu\text{mol/L}$), 10.9% ($0.89 \mu\text{mol/L}$) - 7.5% ($2.5 \mu\text{mol/L}$), respectively.

TAC and TOC analyses: Total antioxidant capacity (TAC) and total oxidant capacity (TOC) were used to measure oxidant antioxidant balance during soccer matches. TAC (mmol Trolox Equivalent /L) and TOC ($\mu\text{mol H}_2\text{O}_2/\text{L}$) were estimated using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (RelAssay Diagnostics, Turkey catalog No: RL0017) according to the manufacturer's instructions.

Trace Elements Measurements

Plasma Copper, Iron, Zinc Analyses

Atomic absorption spectrophotometer is used to determine the levels of plasma copper (Cu) Iron (Fe) and Zinc (Zn) elements. 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. To verify the assay accuracy, standard solutions were run for every 10 test samples.

Statistical Analyses

All data are expressed as mean±S.E.M. The pre-post exercise values for both exercise were assessed using paired t-test and correlation coefficient (r) was used to assess whether there were significant relationships between plasma levels of trace elements and metabolic and oxidative stress factors.

Results

There was a significant increase in MDA level over the course of three soccer matches: from 0.838 ± 0.16 to 1.06 ± 0.17 in morning ($p<0.0001$), from 0.858 ± 0.13 to 1.12 ± 0.18 afternoon ($p<0.0001$) and from 0.870 ± 0.15 to 1.52 ± 0.22 at night ($p<0.0001$). There were no significant differences between basal MDA levels among three soccer matches. However, increase in MDA levels was significantly higher in night (83%) compared to morning (29%, $p=0.003$) and afternoon (33% $p=0.003$) soccer matches.

TOC was increased significantly in all soccer matches: from 14 ± 2.42 to 18.94 ± 1.98 ($p<0.0001$) in morning, from 14.13 ± 3.60 to 20.84 ± 5.93 ($p<0.0001$) in afternoon and from 17.04 ± 3.44 to 29.86 ± 7.87 ($p<0.0001$) at night. Basal TOC was significantly higher in night compared to morning ($p=0.003$) and afternoon ($p=0.01$) soccer matches. In addition, increase in TOC was significantly higher (75%) compared to morning (37%, $p=0.006$) and afternoon (48%, $p=0.02$) soccer matches.

Conversely, TAC decreased significantly over the course of soccer matches: from 0.993 ± 0.07 to 0.818 ± 0.09 ($p<0.0001$) in morning, from 1.04 ± 0.09 to 0.881 ± 0.11 ($p<0.0001$) in afternoon and from 1.14 ± 0.15 to 0.960 ± 0.10 ($p<0.0001$) at night. Basal TAC was significantly higher in night compared to morning ($p=0.003$) and afternoon ($p=0.02$) soccer matches. There were no statistically differences between percent decreases in TAC among three matches.

Iron levels increased significantly from 5.41 ± 0.07 to 7.50 ± 0.11 (38%, $p<0.0001$), from 5.47 ± 0.18 to 7.53 ± 0.19 (37%, $p<0.0001$) afternoon and from 5.49 ± 0.06 to 7.48 ± 0.11 (36%, $p<0.0001$) at night.

Zinc levels decreased significantly from 1.365 ± 0.20 to 1.023 ± 0.18 (-24%, $p<0.0001$). However, there was no statistically differences in afternoon match (1.177 ± 0.26 to 1.147 ± 0.20 ($p=0.5$)) in Zn levels. In contrast, Zn increased from 0.831 ± 0.21 to 1.044 ± 0.26 (28%, $p=0.005$) at night soccer match.

In morning soccer match, Cu levels increased significantly from 0.196 ± 0.03 to 0.278 ± 0.03 (43%, $p<0.0001$). However, Cu levels did not change significant in afternoon match (0.181 ± 0.04 vs 0.176 ± 0.02 ($p=0.5$)). In contrast, Cu decreased significantly from 0.173 ± 0.02 to 0.142 ± 0.02 (-17%, $p<0.0001$) at night soccer match.

Discussion

Exercise induced muscle activity can impose serious physiological alteration in cardio-respiratory system, metabolic system, oxidant and antioxidant balance. Soccer match which require mixed source of energy supply (anaerobic and aerobic) may have significant effects on metabolic and oxidant stress. Biochemical impact of soccer match on oxidative stress and lipid peroxidation has been shown (14). In night soccer match, we have found significantly higher metabolic stress as determined from higher increases in MDA levels and oxidant-antioxidant imbalance compared to morning and afternoon soccer matches. It is logical to expect different flux in trace element levels between tissue and blood with regarding the time of soccer matches. This is the first study evaluated the effects of soccer match performed at three different

time of day (i.e. morning afternoon and at night) on trace elements including Fe, Cu and Zn in sedentary subjects.

Interestingly, we have found a systematic increase in Zn levels in all subjects in morning soccer match but it decreased at night soccer match (Figure 2). In previous studies, Zn levels response to the soccer match showed varied results with regarding time of day (4). A significant decrease in Zn levels as a result of sweat

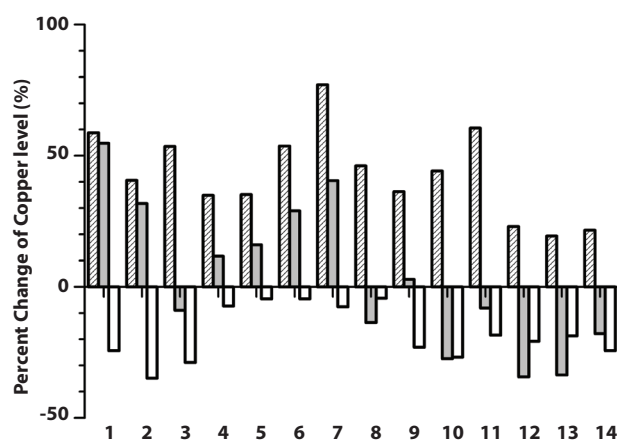


Figure 1. The percent change of copper levels for each individual subject during soccer match performed in morning (backslash column), in the afternoon (grey column) and night (white column)

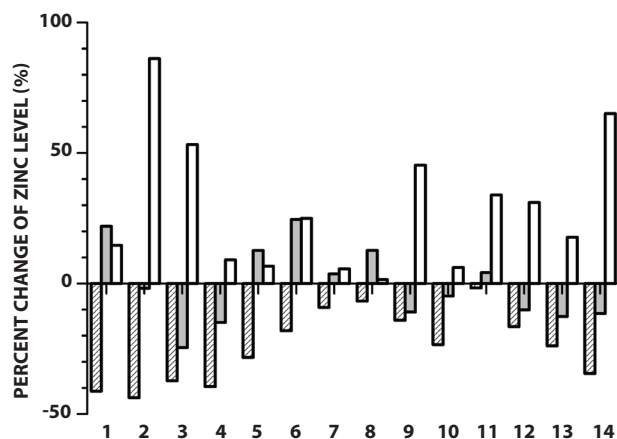


Figure 2. The percent change of Zinc level for each individual subject during soccer match performed in morning (backslash column), in the afternoon (grey column) and night (white column)

after acute exercise has been reported (15,16). However, in afternoon exercise Zn levels did not change despite the highest air temperature among the three soccer matches. During soccer match, the subjects were free of taking fluid that prevents hydration status of the subjects (17).

Zn metabolism could be affected by the exercise type and contribution of muscle amount (17,18). It is known that muscle system is the source of major Zn stores (19) and exercise induced metabolic stress could have a significant effects on increased blood Zn levels (20-22). We have found a systematically decreases in Zn levels in all subjects in morning soccer match (23,24). However, Zn levels did not change significantly in afternoon soccer match (4,25). In the present study, there was marked increase in lipid peroxidation as determined by an 83% increase in MDA and oxidative stress as determined by a 75% increase in TOC during night time soccer match. Increased Zn Levels during night time soccer matches could be results of higher increase in metabolic stress (20,26,27). It is also been considered that functions of zinc transporter proteins could be responsible with the differences in Zn variation with regarding the different exercise times of day (28). In the present study we did not found any correlation between change in MDA and TAC, reflecting metabolic and oxidative stress conditions, and Zn levels in all soccer matches in sedentary subjects. However, a positive correlation between increases in Zn and exercise induced stress hormones variations in well trained athletes (3).

The role of Cu and Zn in the defence of oxidative stress is known. Interestingly, we have found contradictory response of Cu and Zn levels between soccer matches performed in morning and at night (Figure 3) (29). Plasma Cu levels showed different response to the soccer matches which is increased significantly in morning, unchanged in afternoon and decreased significantly at night soccer match (Figure 3). An increase in Cu levels after acute physical exercise has been reported (4,30,31). An marked increases in urinary Cu excretion after physical exercise has been shown (30,32).

Fe is an important trace elements in regulation of body energy metabolic functions (33) and exercise performance capacity (34). In the present study,

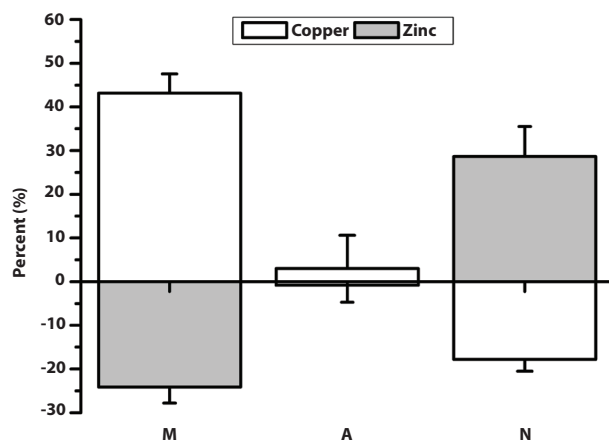


Figure 3. The mean (\pm SD) values for the percent change of copper (white column) and zinc (grey column) for soccer match performed in morning (M), in afternoon (A) and at night (N) (n=14)

soccer match caused significant increase in Fe levels in all subjects. This finding is in agreement with other studies investigating the influence of acute exercise on Fe levels (4,35) Considering the relationships between oxidative stress and Fe levels (9), we could not find any additional impact of night soccer matches on Fe levels compared to morning and afternoon matches. Exercise induced oxidative damage has been shown to increase free iron levels (36). However, a transient decrease in Fe level after soccer match has also been reported (37).

Soccer matches induced metabolic stress has great impacts on trace elements and oxidant balance in untrained subjects. In the present study, we could not find a statistically significant correlation between change in exercise induced metabolic stress parameters and trace elements levels. However, during night time soccer match, observation of higher percentage increase oxidative and metabolic stress as reflected by an increased MDA and TOC levels, decrease in antioxidant level reflected by decrease in Cu levels and increased muscle injury as reflected by increased Zn levels in sedentary subjects should be considered as a hazardous consequence on body health.

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

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