

Serum paraoxonase activity and lipid hydroperoxide levels in adult football players after three days football tournament

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Abstract

Background: It has been suggested that physical activity is an important factor in the prevention and treatment of cardiovascular diseases. Low serum paraoxonase-1 (PON1) activity is with an associated risk of atherosclerotic disease.

Objectives: In this study, we aimed to investigate serum PON1 activity and lipid hydroperoxide (LOOH) levels in adult football players after three days football tournament.

Methods: Twenty-three adult male football players and 23 sedentary male subjects after three days football tournament were enrolled. Serum paraoxonase, arylesterase activities and LOOH levels were determined.

Results: Serum paraoxonase and arylesterase activities were significantly higher in football players than sedentary subjects (all, $p < 0.05$), while LOOH levels were significantly lower ($p < 0.05$). Serum LOOH levels were inversely correlated with paraoxonase and arylesterase activities ($r = -0.552$, $p < 0.001$; $r = -0.812$, $p < 0.001$; respectively) in adult football players.

Conclusion: Our data show, for the first time, that physical activity is associated with increased PON1 activity and decreased oxidative stress after three days football tournament. In addition, physical activity for a healthy life is important in increasing serum PON1 activity, and this may play a role in the prevention of atherosclerosis.

Key words: Football players, physical activity, PON1 activity, lipid hydroperoxide

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Introduction

Oxidative stress is defined as an increase in reactive oxygen species (ROS) production resulting from a deterioration of the balance between oxidants and antioxidants in the body¹. ROS may be generated as observed during aerobic endurance stress. The major source of ROS is thought to be the mitochondria of active working muscles². In this respect, the balance between free radical generation and antioxidant activity plays an important role in the pathogenesis of coronary heart disease.

It is generally accepted that sedentary lifestyle is related to an increased risk of coronary heart disease. In contrast, regular physical activity has been identified as a protective factor against the occurrence and progression of coronary heart disease³. On the other hand, it has been reported that acute exercise is considered to be a major source of free radical generation, oxidative stress, and lipid peroxidation⁴.

Paraoxonase-1 (PON1) is calcium-dependent esterase in human serum that is synthesized

in the liver⁵. Serum PON1 catalyzes the hydrolysis of many organophosphates and aromatic carboxylic acid esters⁶. PON1 is one of the antioxidant enzymes that are associated with high-density lipoprotein (HDL) to prevent oxidative modification of low density lipoprotein (LDL). Therefore, PON1 plays an important role in the protection against atherosclerosis by preventing oxidative modification of serum lipoproteins⁷. Furthermore, previous studies indicated that oxidative stress decreased PON1 activity and down-regulated the serum expression of PON1^{8,9}. Moreover, it has been recently reported that PON1 activity can influence several factors- such as diet, lifestyle, and environmental factors^{10,11}. Moreover, Manresa et al.¹² described the association between PON polymorphism, gender, and exercise. Furthermore, it has been suggested that low serum PON1 activity is significantly associated with an increased risk of atherosclerotic disease¹³.

The effects of physical exercise on PON activity have been investigated in a clinical³ study. Recently, several studies found that serum PON1 activity was increased in adolescent wrestlers¹⁴ and adolescent athletes¹⁵.

To the best of our knowledge, the serum PON1 activity and lipid hydroperoxide levels in adult football players have not yet been reported.

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Therefore, we investigated the serum PON1 activity and lipid hydroperoxide (LOOH) levels as a marker of oxidative stress in adult football players after three days football tournament.

Methods

Subjects

A group of 46 healthy male subjects, 23 adult football players and 23 sedentary controls were included in this study. 23 adult football players were selected among Yuzuncu Yil University, Physical Education and Sports students.

Adult football players were asymptomatic with an unremarkable medical history and a normal physical examination. None of the adult football players had diabetes mellitus, hyperlipidemia, hypertension, coronary artery disease, and psychiatric, metabolic, hepatic or renal disease. Adult football players were non-smokers. None of the subjects reported the use of anti-inflammatory drugs or a regular antioxidant vitamin supplements including vitamin E or C. None of the subjects was following a special diet.

Control subjects were young men who had a sedentary lifestyle and did not regularly participate in physical activity. Control subjects underwent routine physical and laboratory evaluations to ensure that none had diabetes mellitus, hyperlipidemia, hypertension, coronary artery disease, smoking, usage of supplemental vitamins, psychiatric, metabolic, hepatic or renal disease.

All subjects were informed about the study and they gave their informed written consent prior to the beginning of the study. The protocol of the study was conducted in accordance with the Helsinki Declaration as revised in 2000 and was approved by the local ethics committee.

Blood samples

After three days football tournament, blood samples were collected at overnight fasting period, between 08:00 h and 10:00 h from an antecubital vein. Blood samples were collected into empty tubes and immediately stored at 4°C. The serum samples were then separated from the cells by centrifugation at 3,000 rpm for 10 min. The serum samples were stored in plastic tubes at -80°C and used for the analysis of the PON1 activity and LOOH levels.

Measurement of paraoxonase and arylesterase activities

Paraoxonase and arylesterase activities were measured using commercially available kits (Relassay, Gaziantep, Turkey). PON1 activity was determined using two different substrates.¹⁶ First, the rate of hydrolysis of paraoxon was measured by monitoring the increase in absorbance at 412 nm and 25°C due to the formation of p-nitrophenol. PON1 activity assays were carried out in the absence of basal activity and the presence of NaCl (salt-stimulated activity). The serum sample to be tested was added to a cuvette containing 1.0 mmol paraoxon, 1.0 mmol CaCl₂ and 50 mmol glycine/NaOH buffer at pH 10.5. The amount of p-nitrophenol generated was calculated from the molar extinction coefficient at pH 10.5, which was 18 290/mol per centimetre. PON1 activity was expressed as U/L and defined as 1 mmol p-nitrophenol generated per minute under well-established conditions. Second, the PON1 activity was measured in an arylesterase assay using phenyl acetate as substrate. In 10 mL of prediluted (1:3) serum samples were added to 1.5 mL reaction mixture containing 1.0 mmol phenylacetate, 9.0 mmol Tris-HCl buffer, and 0.9 mmol CaCl₂ at pH 8.0 and 25°C. Enzymatic activity was calculated from the molar extinction coefficient 1310/mol per centimetre. Arylesterase activity is expressed as kU/L and defined as 1 mmol phenol generated per minute under the conditions.¹⁷ Blanks without enzyme were used to correct for spontaneous hydrolysis of both substrates.

Measurement of lipid hydroperoxide levels

Serum LOOH levels were measured with ferrous ion oxidation-xylene orange assay. The principle of the assay depends on the oxidation of ferrous ion to ferric ion via various oxidants and the produced ferric ion is measured with xylene orange. LOOHs are reduced by triphenyl phosphine (TPP), which is a specific reductant for lipids. The difference between with and without TPP pretreatment gives LOOH levels.¹⁸

Other parameters

The levels of triglyceride (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), and LDL-cholesterol (LDL-C) were determined using commercially available assay kits (Abbott®) with an autoanalyzer (Aeroset®, Abbott®).

Statistical analysis

The results were expressed as the means and their standard deviation (mean±SD). Nonparametric continuous variables were compared by the Mann-Whitney U-test. Parametric variables were compared using Student's test. Correlation analyses were performed using Pearson's correlation test. The results were considered to be statistically significant when p value was less than 0.05. The data were analyzed using the SPSS® for Windows computing program (Version 11,0).

Results

Demographic characteristics of groups are presented in table 1. There were no significant differences between the groups in terms of age and body mass index (p>0.05) (table 1).

Serum TG, TC and LDL-C levels were significantly lower in adult football players compared to in sedentary subjects (all, p<0.05), while the HDL-C levels were significantly higher (p<0.05) (table 1).

Serum paraoxonase and arylesterase activities were significantly higher in adult football players than in sedentary subjects (all, p<0.05), while LOOH levels were significantly lower (p<0.05) (table 2).

Serum LOOH levels were inversely correlated with paraoxonase and arylesterase activities (r=-0.552, p<0.001; r=-0.812, p<0.001; respectively) in adult football players.

No correlation was observed between the lipids parameters, and serum LOOH levels, paraoxonase and arylesterase activities (all, p>0.05).

Table 1: Demographic characteristics of the two groups in this study

Parameters	Football players (n=23)	Controls (n=23)	p
Age (years)	21±1	22±1	ns
Body mass index (kg/m ²)	21.5±0.3	21.2±0.4	ns
TG (mg/dl)	87.32±36.11	117.74±42.31	<0.05
TC (mg/dl)	126.94±34.02	168.55±36.82	<0.05
HDL-C (mg/dl)	52.16±12.24	42.10±11.24	<0.05
LDL-C (mg/dl)	56.84±15.85	103.80±17.88	<0.05

Values are mean ± SD ns = non significant TG: Triglyceride
TC: Total cholesterol HDL-C: High-density lipoprotein-cholesterol
LDL-C: Low-density lipoprotein-cholesterol

Table 2: PON1 activity and oxidative stress levels of the two groups

Parameters	Football players (n=23)	Controls (n=23)	p
Paraoxonase (U/L)	167.41±49.05	127.93±27.70	p<0.05
Arylesterase(kU/L)	157.12±23.84	130.03±36.71	p<0.05
LOOH(μmol/L)	5.33±1.35	6.46±1.17	p<0.05

Values are mean ± SD LOOH: Lipid hydroperoxide

Discussion

In the present study, we measured serum PON1 activity and LOOH levels as a marker of oxidative stress in adult football players after three days football tournament. We observed that adult football players had increased paraoxonase and arylesterase activity along with decreased LOOH levels. In addition, we found a negative correlation between paraoxonase, arylesterase activity and LOOH in adult football players. To the best of our knowledge, this study is the first to show that physical exercise is associated with decreased LOOH along with increased PON1 activity.

ROS are physiological products of aerobic metabolism. They contribute to the stimulation of anti-oxidative processes¹⁹. It has been shown that regular physical activity is an important factor in the prevention and treatment of cardiovascular diseases²⁰. Regular physical exercise is a protective factor against cardiovascular diseases and enhances antioxidant systems, whereas acute exercise appears to be a major source of increased oxidative stress²⁰. It has been reported that exercise training appears to enhance antioxidant levels²¹ and reduce lipid peroxidation levels⁴, improving serum oxidant status in humans²².

The useful effect of exercise on health has been well known for centuries. Regular exercise is linked to reduced risk of developing obesity, hypertension, coronary artery disease and diabetes, and helps prevent premature death²³. It has been shown that regular physical activity has beneficial effects on lipoprotein metabolism, including a decrease in plasma triglyceride levels and an increase in HDL-cholesterol levels, which bears antioxidant properties²⁴. In the present study, we observed that adult football players had decreased serum TG, TC and LDL-C levels compared to in sedentary subjects, while the HDL-C levels were significantly higher.

Paraoxonases are a new addition to the antioxidant enzyme family. Several lines of evidence suggest that PON1 is responsible for the antioxidant properties of HDL on LDL particle^{25,26}. Reduced PON1 activity has been reported in various diseases such as diabetes mellitus²⁷, atherosclerotic heart disease²⁸ and hypercholesterolemia²⁹ and in patients who are under increased oxidative stress³⁰. PON1 is also one of the antioxidants that act as an enzymatic defense against lipid hydroperoxides³¹. In addition, oxidative stress has been reported to affect the expression and activity of PON1^{8,9}.

Several studies have investigated PON1 activity in many different conditions^{3,14,15}. Tomas et al³ assessed the effects of a single bout of exercise on PON1 activity in humans. Hamurcu et al¹⁴ demonstrated higher serum PON1 activity in wrestlers than sedentary. Cakmak et al¹⁵ found higher serum PON1 activity athletic adolescents with regular physical activity than in controls. On the other hand, a previous study reported significantly increased PON1 activity values in well-trained rugby players compared with sedentary controls³². In addition, regular aerobic exercise in well-trained amateur athletes has been associated with an increase in PON1 activity³³. To the best of our knowledge, the serum PON1 activity and lipid hydroperoxide levels in adult football players have not yet been reported.

Although PON1 activity and concentration are determined genetically, several factors such as diet, lifestyle, and environmental factors, can influence PON1 activity and/or concentration. Degraded cooking oil has been reported to decrease serum PON1 levels in humans³⁴.

The mechanism of the observed increase in serum PON1 activity in football players remains unclear. This increase could be related to decrease lipid peroxidation because oxidized lipids are reported to inhibit PON1 activity. Furthermore,

PON1 is an HDL-associated enzyme, and PON1 activity is positively correlated with HDL-C levels³⁵. Indeed, in this study, adult football player's subjects had higher HDL-C levels than controls. However, in contrast to a previous study³⁶, there was no correlation between PON1 activity and HDL-C.

Conclusion

Our data show, for the first time, that physical activity is associated with increased PON1 activity and decreased oxidative stress after three days football tournament. These results indicate that physical activity for a healthy life is important for increasing serum PON1 activity and that this may play a role in the prevention of atherosclerosis. Further studies with larger populations are needed to explore this relationship.

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