Soccer players under regular training show oxidative stress but an improved plasma antioxidant status

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ABSTRACT

Physical activity is known to induce oxidative stress in individuals subjected to intense exercise. In this study, we investigated the lipoprotein profile and the plasma antioxidant status in a group of soccer players engaged in a regular training programme. As was expected for aerobic exercise, high-density lipoprotein-cholesterol (HDL-C) and HDL$_3$-C levels were significantly increased in the sportsmen ($P < 0.05$). Total plasma antioxidant capacity was $25\%$ higher in sportsmen than in controls ($P < 0.005$). Accordingly, plasma hydrosoluble antioxidant levels (ascorbic acid and uric acid) were found to be significantly elevated in the soccer players ($P < 0.005$). In addition, these subjects showed high concentrations of $\alpha$-tocopherol in plasma compared with controls ($P < 0.005$). Furthermore, an increase in plasma superoxide dismutase activity was also observed in relation to exercise ($P < 0.01$). The elevation in plasma activities of antioxidant enzymes and the higher levels of free radical scavengers of low molecular mass may compensate the oxidative stress caused by physical activity. High levels of high-density lipoprotein in plasma may offer additional protection by inhibiting low-density lipoprotein oxidation and thus liposoluble antioxidant consumption. Therefore, soccer players under regular training show an improved plasma antioxidant status in comparison to sedentary controls.

INTRODUCTION

It is now accepted that oxidative stress may be implicated in the aetiology of atherosclerosis. Atherosclerosis risk is determined not only by the absolute levels of atherogenic lipoproteins, but also by the relative tendency of such particles to undergo oxidation [1,2]. Low-density lipoprotein (LDL), which is recognized as having a high atherogenic capacity, is susceptible to oxidative processes initiated by oxygen free radicals. Conversely, high-density lipoprotein (HDL) has been traditionally considered to play an anti-atherogenic role due to its capacity to transport excess cholesterol from peripheral tissues to the liver for excretion [3]. Furthermore, it has recently been reported that HDL could protect LDL from oxidative damage [4], and experiments carried out in vitro have shown that HDL is able to inhibit LDL oxidation by metal ions [5]. It is known that physical activity is one of the factors liable to increase HDL plasma levels [6].

Physical exercise is characterized by an increase in oxygen consumption by the whole body and particularly by the muscle. This increase in oxygen uptake is associated with a rise in the production of reactive oxygen species (normally 2–5% of the total oxygen consumption). The high production of reactive oxygen species may be responsible for a series of physiological and

Key words: antioxidant capacity, ascorbic acid, exercise, high-density lipoprotein, lipoprotein, oxidative stress, soccer, superoxide dismutase, uric acid, vitamin E.
Abbreviations: HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol.
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biochemical changes that occur during exercise [7]. It has been reported that strenuous physical exercise produces a decrease in antioxidant levels and an increase in the markers of lipid peroxidation in target tissues and blood [8–10]. On the other hand, antioxidant defences appear to be modulated by the state of physical training. Several studies have shown that training increases the glutathione (GSH) pool and catalase activity in rat skeletal muscle [11] and catalase and superoxide dismutase activities in human muscle [12].

Even though acute physical exercise is known to produce oxidative stress, individuals who follow a regular training programme could have an improved antioxidant status. The aim of this work was to evaluate the changes in the lipid and lipoprotein profile and in plasma antioxidant levels in a group of sportsmen (soccer players) engaged in a physical training programme. Details of the lipid and lipoprotein profile and measurements of total plasma antioxidant capacity, hydrosoluble antioxidants (ascorbic acid, uric acid and bilirubin levels), liposoluble antioxidants (α-tocopherol levels) and superoxide dismutase activity are provided.

**METHODS**

**Subjects and samples**

Two groups comprising 30 sportsmen and 12 sedentary control subjects were studied. All subjects were male. The sportsmen were soccer players engaged in a controlled physical training programme that consisted of 20 h of training and six soccer matches per week for at least 1 year. Sport was practised at sea level. The control subjects were medical students who had a sedentary lifestyle and did not practise any sport regularly. All of the subjects selected were healthy without familial or personal history of diabetes or dyslipidaemia and normal thyroid, hepatic and renal functions. None of the subjects was taking any drug known to affect lipid and lipoprotein metabolism. Special care was taken to exclude subjects who were taking anabolic drugs, vitamins or other antioxidants, or who were smokers. Ethanol intake was considerably below 50 g per week in all the participants. None of the subjects was following a special diet. A face to face interview was conducted with every participant and a detailed food frequency questionnaire was completed in order to obtain information about their dietary habits [13]. The quality, quantity and frequency of consumption of red meat, chicken, fish, eggs, vegetables, fruits, milky products and soft drinks was similar in all of the subjects. They followed a typical Argentine diet which mainly consisted of: red meat, seven times a week; chicken, three times a week; fish, once a week; and floury foods, three times a week. Vegetables in low quantities and not more than one fruit were consumed at each meal.

The sportsmen and controls were all sampled within the same 2 weeks during spring. After a 12-h overnight fast, venous blood was drawn from the antecubital vein. The samples were collected into clean tubes for analyses of lipids and lipoproteins, uric acid and total bilirubin, and into heparin-containing tubes for determination of total antioxidant potential, ascorbic acid and α-tocopherol and evaluation of superoxide dismutase activity. Samples were maintained in a cold chamber (4 °C) for 1 h until separation by centrifugation at 1500 g for 15 min at 4 °C, and immediately used for lipid and lipoprotein and ascorbic acid determinations. Aliquots were stored at −80 °C for the other studies, which were carried out within 30 days. The study protocol was in accordance with the policy statements of the American College of Sports Medicine and was approved by the Ethical Committee of the School of Pharmacy and Biochemistry, University of Buenos Aires. Informed consent was obtained from all participants.

**Lipid and lipoprotein profile**

Total cholesterol and triacylglycerol levels were quantified by standardized enzymic methods (Boehringer Mannheim, Germany) in an ABA VP autoanalyser. Within-run coefficients of variation were 1.11% and 1.32% respectively. Between-day coefficients of variation were 1.52% and 2.44% respectively. Laboratory bias was −1.7% for total cholesterol and 1.1% for triacylglycerols. HDL was isolated in the supernatant obtained after precipitation of apolipoprotein B-containing lipoproteins with 20 g/l dextran sulphate (50 kDa) and 1.0 mmol/l MgCl₂ [14]. HDL₃ was separated by precipitation of the supernatant containing total HDL with 40 g/l dextran sulphate and 2.0 mmol/l MgCl₂ [14]. Cholesterol (C) in total HDL and HDL₃ fractions was determined by a standardized enzymic method (Boehringer Mannheim, Germany). Within-run and between-day coefficients of variation for HDL-C were 3.2% and 3.8% respectively. Laboratory bias was −2.0%. HDL₃-C was calculated as the difference between HDL-C and HDL₃-C. The LDL-C level was determined as the difference between total cholesterol and the cholesterol contained in the supernatant obtained after selective precipitation of LDL with 10 g/l poly(vinyl sulphate) in poly(ethylene glycol) (600 Da; 2.5% w/v; pH = 6.7) [15]. Within-run and between-day coefficients of variation were 4.7% and 5.0% respectively. Results are expressed in mmol/l.

**Total antioxidant potential**

Total antioxidant potential or total antioxidant capacity was measured by chemiluminescence. The reaction medium consisted of 20 mmol/l 2,2’-azo-bis(2-amidinopropane) and 40 μmol/l luminol. 2,2’-Azo-bis(2-amidinopropane) is a source of free radicals and it reacts with luminol yielding chemiluminescence, which was
measured in an LKB liquid scintillation counter. The addition of 10 μl of plasma decreases chemiluminescence to basal levels, for a period proportional to the amount of antioxidants present in plasma, until luminol radicals are regenerated. The system was calibrated with Trolox (vitamin E hydrosoluble analogue). Results are expressed as μmol Trolox/l [16].

**Determination of plasma antioxidant levels**

Ascorbic acid concentration was quantified by HPLC with electrochemical detection using an ESA Coulochrome II electrochemical detector (Bedford, MA, U.S.A.) at an applied oxidation potential of 0.4 V [17]. Plasma samples were previously treated with 200 μl of 10% (w/v) metaphosphoric acid. After centrifugation at 1500 g for 10 min, 300 μl of 0.8% (w/v) metaphosphoric acid was added to 90 μl of the supernatant. Results are expressed in μmol/l.

Uric acid and total bilirubin levels were measured by standardized enzymic methods (Boehringer Mannheim, Germany) in an ABA VP autoanalyser. Results are expressed in μmol/l.

α-Tocopherol was quantified by reverse-phase HPLC with electrochemical detection using a Bioanalytical Systems (West Lafayette, IN, U.S.A.) amperometric detector with a glassy-carbon working electrode at an applied oxidation potential of 0.6 V [18]. Plasma samples were extracted with 1 ml of ethanol and 3 ml of hexane. After centrifugation at 1500 g for 10 min, the upper phase was removed and evaporated to dryness under nitrogen. Samples were dissolved in 0.3 ml of methanol/ethanol (1:1, v/v). Results are expressed in μmol/l.

**Superoxide dismutase activity**

Superoxide dismutase activity was determined spectrophotometrically in plasma samples by measuring the inhibition of the rate of autocatalytic adrenochrome formation at 480 nm in a reaction medium containing 1 mmol/l adrenaline and 50 mmol/l glycine/NaOH (pH = 10.2). The enzymic activity is expressed as superoxide dismutase units per litre of plasma. One unit is defined as the amount of enzyme that inhibits the rate of adrenochrome formation in 50% [19].

**Data and statistical analysis**

Data are presented as means ± S.E.M. When data did not follow the Gaussian distribution, the Mann–Whitney non-parametric test (U-test) was used to compare the different groups. Differences were considered significant at P < 0.05 in the bilateral situation.

**RESULTS**

Anthropometric characteristics of sportsmen and control subjects are shown in Table 1. Both groups were of similar age, body mass index and waist/hip ratio.

| **Table 1 Anthropometric characteristics of sportsmen (n = 30) and sedentary controls (n = 12)** |
|-----------------|-----------------|
| **Values are means ± S.E.M.** |
| **Age (years)** | **Sportsmen**  | **Controls** |
| 17.5 ± 0.3      | 18.3 ± 0.3      |
| **Body mass index (kg/m²)** | **Sportsmen**  | **Controls** |
| 20.0 ± 0.3      | 21.4 ± 0.9      |
| **Waist/hip ratio** | **Sportsmen**  | **Controls** |
| 0.80 ± 0.01     | 0.80 ± 0.02     |

| **Table 2 Lipid and lipoprotein levels in sportsmen (n = 30) and sedentary controls (n = 12)** |
|-----------------|-----------------|
| **Values are means ± S.E.M. *P < 0.05 compared with controls by Mann–Whitney U-test. SOD, superoxide dismutase.** |
| **Antioxidant profile in sportsmen (n = 30) and sedentary controls (n = 12)** |
| **Values are means ± S.E.M. *P < 0.005, † P < 0.01 compared with controls by Mann–Whitney U-test. SOD, superoxide dismutase.** |

| **Hydrosoluble antioxidants** | **Sportsmen** | **Controls** |
| 405 ± 17* | 319 ± 11 |
| Ascorbic acid (μmol/l) | 97.7 ± 10.2* | 39.5 ± 15.0 |
| Uric acid (μmol/l) | 327 ± 9* | 247 ± 12 |
| Total bilirubin (μmol/l) | 15.4 ± 1.7 | 12.0 ± 1.7 |

| **Liposoluble antioxidant** | **Sportsmen** | **Controls** |
| α-Tocopherol (μmol/l) | 25.7 ± 0.5* | 22.6 ± 0.5 |

| **Table 3 Antioxidant profile in sportsmen (n = 30) and sedentary controls (n = 12)** |
|-----------------|-----------------|
| **Values are means ± S.E.M. *P < 0.005 compared with controls by Mann–Whitney U-test. SOD, superoxide dismutase.** |
| **Antioxidant profile** | **Sportsmen** | **Controls** |
| 15.8 ± 1.8† | 9.3 ± 0.7 |

Lipid and lipoprotein plasma levels are shown in Table 2. No significant differences were found in triacylglycerol, total cholesterol and LDL-C concentrations between sportsmen and the control group. However, training produced a significant increase of approximately 10% in the amount of cholesterol transported in HDL compared with the control group (P < 0.05). Similar results were found for the subfraction HDL₂ (P < 0.05).

Total antioxidant capacity is considered a valuable measurement of the antioxidant status of biological fluids and tissues [20]. As shown in Table 3, total antioxidant capacity was 25% higher in the sportsmen than in the control subjects (P < 0.005).

Ascorbic acid, uric acid and total bilirubin determinations are shown in Table 3. A significant 3-fold increase was observed in the ascorbic acid levels of
sportsmen compared with the control group \((P < 0.005)\).

The plasma concentration of uric acid was 30% higher in sportsmen than in the non-trained group \((P < 0.005)\), although both values fell within the reference range \((179–417 \mu \text{mol/l})\). No significant changes were found in plasma levels of total bilirubin.

The plasma concentration of \(\alpha\)-tocopherol was also evaluated. Tocopherol plasma levels in the group of sportsmen showed a significant increase of approximately 10% \((P < 0.005)\). The value for the control group was \(22.6 \pm 0.5 \mu \text{mol/l}\) (Table 3).

The activity of superoxide dismutase in plasma is also given in Table 3 for both groups. Superoxide dismutase activity was significantly increased (approximately 52%) in the sportsmen compared with the control group \((P < 0.01)\).

**DISCUSSION**

In this study, a group of young soccer players engaged in a controlled physical training programme showed an improvement in the lipoprotein profile and in plasma antioxidant levels compared with sedentary controls. Overall, these findings suggest a protective response arising from regular physical activity.

In this group of trained subjects, a significant increase in HDL-C levels was found in relation to exercise. The observed data are in agreement with various studies mainly focused on aerobic physical activity [21]. This elevation was attributable to HDL, but the selective increase in this subfraction was not due to an effect of alcohol on lipoprotein metabolism because participants mainly consumed soft drinks. HDL has invariably been considered as a protective factor due to its antiatherogenic capacity and, more recently, to its role in inhibiting LDL oxidation [4]. The mechanism underlying this effect may be associated with the transport and neutralization of highly reactive oxidized lipid species from LDL towards HDL. It has been suggested that interplay between HDL-related hydrosoluble enzymes, HDL-mediated uptake of oxidized cholesteryl esters and HDL-incorporated antioxidants contribute to the protection given by HDL to LDL [5].

It is now accepted that exercise induces oxidative stress, which is defined as an increase above physiological values of the steady-state concentrations of active oxygen species: \(\text{O}_2^−, \text{H}_2\text{O}_2, \text{HO}^−, \text{ROO}^−\) and \(1\text{O}_2\) [22]. The high oxygen consumption and consequent oxidative stress during physical activity causes a series of diverse responses. These may involve both lipid peroxidation of biological membranes, which can lead to extensive cellular damage [23], and also changes in the redox status of the cell thus inducing modifications in the levels of certain antioxidants [24].

In the present work, trained subjects showed increased total antioxidant capacity values, ascorbic acid, uric acid and, \(\alpha\)-tocopherol levels and superoxide dismutase activity in response to the oxidative stress imposed by physical activity. It is noteworthy that in this study we evaluated young sportsmen following a regular training programme, not sedentary individuals subjected to acute physical activity over a certain period of time, as reported by several authors [25,26].

Evaluation of total antioxidant capacity is one of the most common procedures employed to evaluate the hydrosoluble antioxidant status of biological fluids [27,28]. This methodology reflects the capacity of a sample to quench the luminol-derived chemiluminescence and can be related to its ability to trap oxygen free radicals [20]. Therefore, hydrosoluble antioxidants such as ascorbic acid, GSH, uric acid and bilirubin are evaluated as a whole in this determination. The increase in total antioxidant capacity values observed in sportsmen is consistent with the higher plasma levels of ascorbic acid and uric acid.

Ascorbic acid has multiple antioxidant properties, including the ability to regenerate \(\alpha\)-tocopherol by reducing \(\alpha\)-tocopheryl radicals at the surface of cellular membranes. Thus, the high levels of ascorbic acid in sportsmen improve the overall antioxidant status in these subjects. As the individuals in our study were not receiving vitamin C supplementation, the increased levels observed may be attributed to a shift in ascorbic acid from tissue to blood as a result of exercise. These data are in agreement with previous studies carried out in running individuals and sedentary controls [29].

On the other hand, uric acid may act as an antioxidant both by binding iron and copper ions and also by directly scavenging reactive oxygen species [30]. In soccer players, plasma levels of uric acid were significantly higher than in controls, although they remained within the reference values. In addition, uric acid adds to the enhanced antioxidant profile by protecting ascorbic acid from oxidative reactions in plasma [31].

As with ascorbic acid, the increase in \(\alpha\)-tocopherol plasma levels could be due to the mobilization from tissue storage to plasma circulation. The redistribution of vitamin E from tissues to blood was also suggested by Pinccmail et al. [32]. However, the latter study was carried out in men subjected to intense cycling to exhaustion.

In addition to the increase in plasma levels of low-molecular-mass antioxidants, the sportsmen also had a high superoxide dismutase activity in plasma. This finding is in accordance with previously reported data in human and animal models [11,12]. Exercise-induced changes in the redox status of tissues may initiate intracellular signal transduction processes that trigger antioxidant defence protein expression [24].

In summary, high oxygen consumption during physical activity leads to oxidative stress which may be
compensated by higher levels of free radical scavengers of low molecular mass and by the elevated activities of antioxidant enzymes. The redistribution from tissue to plasma and the interaction between different antioxidants may improve the antioxidant plasma status. Furthermore, high levels of HDL in plasma could offer additional protection by inhibiting LDL oxidation and thus lipo-soluble antioxidant consumption. Therefore, subjects under regular training show an overall improved antioxidant status. Thus, these sportsmen are more protected from oxidative-stress-related pathologies such as athero-sclerotic cardiovascular disease.

ACKNOWLEDGMENTS

This work was supported by grants from the University of Buenos Aires (FA 035 and FA 062) and from the Alberto J. Roemmers Foundation. We thank the sportsmen, trainers and managers from Club Atlético Lanús. We are grateful to Professor Oscar Mendez for his personal and scientific interest in this work.

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