Paraoxonase Activity in Athletic Adolescents

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Regular physical activity may play a protective role against cardiovascular disease in adults, and paraoxonase activity may serve to mediate this effect. This study compared paraoxonase activity and that of other antioxidative agents in adolescent athletes compared with inactive youth. Paraoxonase level was $177.32 \pm 100.10$ (U/L) in children with regular physical activity and $98.11 \pm 40.92$ (U/L) in the control group ($P < 0.0001$). The levels of total antioxidative capacity, total oxidative status, oxidative stress index, and lipid hydroperoxide were significantly higher in the athlete group compared with controls ($P < 0.0001$). Paraoxonase activity was found to be greater in adolescent athletes, suggesting that regular exercise might provide a cardio-protective effect by this means.

Coronary heart disease (CHD) is the most common cause of mortality and morbidity worldwide (39). CHD usually affects middle-aged and elderly people, but in recent years, the incidence of CHD in younger individuals has been increasing (18). Atherosclerosis is a process that begins in childhood and shows a long preclinical phase before leading to clinical manifestations, which usually appear in middle age (41). Changes in the peripheral vascular endothelium are the earliest signs of atherosclerosis and CHD (38). This has been demonstrated in asymptomatic children and young adults who are healthy but who also carry risk factors for atherosclerosis (e.g., high cholesterol, smoking, diabetes; 9).

In addition to these classical risk factors, the oxidation of low-density lipoprotein (LDL) plays a role in the impairment of endothelial function and arterial reactivity (38), as was supported by a recent cell culture study demonstrating that even very low levels of oxidized LDL can decrease the generation of nitric oxide (55), an important mediator of coronary vasomotor tone. High-density lipoprotein (HDL) retards the accumulation of lipid peroxides on LDL cholesterol (31). This appears to be due to paraoxonase, an enzyme associated with HDL particles (56). Paraoxonase has two known functions: it contributes to the detoxification of organophosphorus compounds, including the pesticide paraaxon, and it also hydrolyzes lipid peroxides and prevents LDL oxidation (27).

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Regular physical activity (RPA) is involved in reducing the risk of CHD (36), and consequently, physical inactivity has been considered a risk factor for CHD (6). On the other hand, oxidative stress is known to be associated with the development of several chronic diseases, including atherosclerosis (57). The oxidation of LDL components is a cornerstone of atherosclerosis (57). The balance between free radical generation and antioxidant activity is critical in the pathogenesis of CHD. LDL resistance to oxidation, mainly dependent on its antioxidant content and lipid particle composition, is also an important factor limiting this process. One of the favorable effects of RPA may be modification of the balance between oxidants and antioxidants, thus causing an increase in endogenous antioxidant activity and LDL resistance to oxidation. On the other hand, acute physical activity (PA) increases oxygen uptake and free radical production and consequently may also induce lipid peroxidation (10).

In this respect, the balance between free radical generation and antioxidant activity seems to play a key role in the pathogenesis of CHD. Paraoxonase is an antioxidant enzyme associated with HDL that contains apolipoprotein (apo) A-I (7) and apo J (7). Several emerging lines of evidence suggest that paraoxonase is responsible for the antioxidant properties of HDL on LDL particles (32).

Paraoxonase, synthesized in the liver and in serum, is located on HDL. The serum HDL concentration is inversely correlated with atherosclerosis risk (34). Paraoxonase [PON 1; EC 3.1.8.1] aryldialkyl phosphatase has been studied extensively in the field of toxicology because it hydrolyzes organophosphate compounds, which are used as insecticides and nerve gases (28). This mechanism continues to be the subject of considerable debate. However, recent studies have suggested mechanisms that are more diverse. HDL protects against oxidative modification of LDL, which is believed to be central to the initiation and progression of atherosclerosis (49). Some studies have shown that serum paraoxonase activity is reduced in diabetes and familial hypercholesterolemia (1), diseases that are associated with accelerated atherogenesis. HDL-C is a complex particle composed of a hydrophobic core, which in normolipidemic states consists mostly of esterified cholesterol (50). The surface of the HDL particle is covered with multiple proteins that play critical roles in determining the overall cardioprotective effects of the lipoprotein. One of these proteins is paraoxonase, an enzyme capable of protecting the HDL particle from oxidative modification, thereby preserving its cardioprotective effects. Both genetic and biochemical studies in animal models and in humans have suggested that increased serum paraoxonase activity is atheroprotective, decreasing the risk for major adverse cardiovascular events (47).

The paraoxonase activity, lipid levels, and the oxidative status of children with RPA as well as controls were analyzed in this study.

**Materials and Methods**

**Subjects**

The study group consisted of 64 adolescent amateur basketball players (57 boys, 7 girls) who had regularly undertaken 2 hr of training per day at least 3 days per week for at least 2 years. The control group consisted of 32 healthy adolescents of similar age (24 boys, 8 girls) who did not participate in sports. They were selected from healthy adolescents who presented to our pediatric clinic. The girls in both the study and control groups were postmenarcheal. Both groups were instructed not
to eat, drink, or take any antioxidant medicine for 3 hr before sample collection. All participants were healthy, with no familial or personal history of diabetes or dyslipidaemia and with normal thyroid, hepatic, and renal functions. Subjects were withdrawn from the study if there was any indication of cardiovascular disease, physical discomfort, or chronic illness, or if exercise was likely to trigger asthma. A face-to-face interview was conducted with the participants, and a detailed food frequency questionnaire was completed to obtain information about their dietary habits.

A food consumption questionnaire was used to record the consumption frequency and daily consumption of different types of food of the participants (35). The types of food consumed by the subjects were recorded on the forms as portion or as weight for every meal, daily, 1–3 times per week, 3–5 times per week, once per two weeks, and once a month. These food types were categorized as dairy products, eggs, meat, grains, bread and cereals, fresh fruit and vegetables, oil, sugar, desserts, drinks, and subgroups of each category. The daily energy amounts obtained from different food groups by the study group and control group were calculated and recorded using the data obtained from the food consumption questionnaire.

The subjects were not taking any drugs known to affect lipid or lipoprotein metabolism. Special care was taken to exclude subjects who were taking anabolic drugs, vitamins, or other antioxidants, or who were smokers. None of the subjects had a special diet.

The amount of sport undertaken weekly, the number of years engaged in this sport, dietary habits, personal and familial health history, socioeconomic position, smoking habit or exposure to smoking, and use of vitamins or medications were recorded.

The quality, quantity, and frequency of consumption of red meat, chicken, fish, eggs, vegetables, fruits, milk products, and soft drinks were similar for all subjects. The economic and sociocultural status were similar in the study and control groups. The dietary habits of both groups were also similar.

Before the study, all subjects underwent medical examination, and their medical and sporting histories were recorded. The study protocol was approved by the local ethics committee. The details of the study were explained to the participants before enrollment and all parents provided informed consent.

Measurements

The ages of the participants were recorded, height was measured to 0.1 cm, and weight was measured to 0.1 kg in both groups. Respiratory function was measured by a flow tester screen mark spirometer, and the systolic and the diastolic blood pressure were measured using a stethoscope and sphygmomanometer. Heart rate was measured from the wrist radial artery by counting the pulse for 15 s then multiplying by 4 to give the number of beats per minute. Spirometry was carried out to identify and exclude subjects with asthma.

Samples

Blood samples obtained following an overnight fast were collected into empty tubes and immediately stored on ice at 4 °C. The plasma samples were then separated from the cells by centrifugation at 3000 rpm for 10 min, and lipid parameters and enzymes activities were measured immediately. Plasma samples were stored at -80 °C until analysis.
Measurement of Paraoxonase and Arylesterase Activities

Paraoxonase and arylesterase activities were measured using paraoxon and phenylacetate as substrates. The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase in absorbance at 412 nm at 37 °C. The amount of p-nitrophenol generated was calculated from the molar absorptivity coefficient at pH 8, which was 17000 M⁻¹ cm⁻¹ (13). Paraoxonase activity was expressed as U/L plasma. Phenylacetate was used as a substrate to measure the arylesterase activity. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol, 1310 M⁻¹ cm⁻¹. One unit of arylesterase activity was defined as 1 mmol phenol generated per minute under the above conditions and expressed as U/L plasma (19). Paraoxonase phenotype distribution was determined by a double substrate method that measures the ratio of paraoxonase activity (with 1 M NaCl in the assay) to arylesterase activity, using phenylacetate (13). The coefficient of variation for individual plasma samples was less than 2%.

Measurement of Total Antioxidative Capacity (TAC)

The total antioxidant capacity of the plasma was determined using a novel automated measurement method developed by Erel (14). This method involves production of biologically potent hydroxyl radicals. In this assay, the antioxidative effects of the sample against potent free radical reactions initiated by the produced hydroxyl radicals are measured. The assay has excellent precision with coefficients of variation less than 3%. The results are expressed as mmol Trolox equivalent/L.

Measurement of Total Peroxide Concentration (LOOH)

The total peroxide concentrations of the plasma samples were determined using the FOX2 method (33) with minor modifications (20). The FOX2 test system is based on oxidation of ferrous ions to ferric ions by various types of peroxide contained in the plasma samples to produce a colored ferric-xylenol orange complex the absorption of which can be measured. The FOX2 reagent was prepared by dissolving ammonium ferrous sulfate (9.8 mg) in 250 mmol H₂SO₄ (10 mL) to give a final concentration of 250 mmol ferrous ions in acid. This solution was then added to 90 mL of HPLC-grade methanol containing 79.2 mg of butylated hydroxytoluene (BHT). Finally, 7.6 mg of xylenol orange was added and stirred to make the final working reagent (250 mmol ammonium ferrous sulfate, 100 mmol xylenol orange, 25 mmol H₂SO₄, and 4 mmol BHT in 90% vol/vol methanol in a final volume of 100 mL). The blank working reagent contained all the components of the previous reagent except ferrous sulfate. Aliquots (200 Al) of plasma were mixed with 1800 Al FOX2 reagent. After incubation at room temperature for 30 min, the vials were centrifuged at 12000 × g for 10 min. Absorption of the supernatant was then determined at 560 nm. The total peroxide content of the plasma samples was determined as a function of the absorption difference between the test and the blank tubes using a solution of H₂O₂ as a standard. The coefficient of variation for individual plasma samples was less than 5%.
Measurement of Total Oxidant Status (TOS)

The total oxidant status (TOS) of plasma was determined by using a novel automated measurement method as described previously (15). Oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundant in the reaction medium. The ferric ion produced a colored complex with xylenol orange in acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with \( \text{H}_2\text{O}_2 \) and the results were expressed in terms of micromolar \( \text{H}_2\text{O}_2 \) equivalents per liter (mmol \( \text{H}_2\text{O}_2 \) equivalent/L). The coefficient of variation for individual plasma samples was less than 3%.

Oxidative Stress Index (OSI)

The percentage ratio of total peroxide level to TAC level was taken as the oxidative stress index (OSI; 50). To perform the calculation, the unit of TAC, mmol Trolox equivalent/L, and the OSI value was calculated using the following formula; OSI = [(Total peroxide, mmol/L)/(TAC, mmol Trolox equivalent/L)/100].

Measurement of Lipid Profiles

Plasma triglyceride, total cholesterol, LDL, HDL, and very low-density lipoprotein (VLDL) were measured using an automated chemistry analyzer (Aeroset; Abbott Laboratories, Abbott Park, IL) using commercial kits (Abbott Laboratories).

Statistical Analysis

All data are expressed as means ± standard deviation (SD). Qualitative variables were assessed by the Chi-square test. Correlation analyses were performed using Pearson’s correlation test or Spearman’s correlation test. The differences between the control and patient groups were analyzed by unpaired \( t \) test or Mann–Whitney \( U \) test, and \( P < 0.05 \) was considered to indicate significance. Data were analyzed using SPSS for Windows v. 11.5 (SPSS Inc., Chicago, IL).

Results

The demographic characteristics of the study population are shown in Table 1. Paraoxonase activity as well as arylesterase activity in the subjects with RPA was significantly greater than control subjects (\( P < 0.0001 \); Figure 1, Table 2). No correlations were found between the paraoxonase / arylesterase activities and TAC, TOS, LOOH, OSI, TG, TC, HDL-C, or LDL-C in the study group or the controls. There were also no correlations between the paraoxonase / arylesterase activities and age, height, or weight in the study group or the controls. The levels of total TAC, TOS, and OSI were significantly higher in the athlete group than in the control group (Table 2). There were no statistically significant differences between the two groups in the respiratory function tests, arterial blood pressure, pulse, or respiratory rate. No significant differences were found between the two groups in the values of triglyceride, cholesterol, HDL-cholesterol, LDL-cholesterol, or VLDL-cholesterol (Table 3).
Table 1  Demographic and Clinical Characteristics of RPA Groups and Control Group

<table>
<thead>
<tr>
<th>Variables</th>
<th>RPA group (n = 64)</th>
<th>Control group (n = 32)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>57/7</td>
<td>24/8</td>
<td>0.07, OR: 0.36 (0.12–1.13)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>15.2 ± 1.9</td>
<td>14.8 ± 1.5</td>
<td>0.34</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.5 ± 11.5</td>
<td>162.2 ± 8.5</td>
<td>0.30</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54.2 ± 12.5</td>
<td>53.1 ± 10.4</td>
<td>0.67</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.8 ± 3.2</td>
<td>20.0 ± 2.4</td>
<td>0.75</td>
</tr>
</tbody>
</table>

*Note.* Values are expressed as means ± SD

![Paraoxonase levels according to group.](image)

Paraoxonase activity was significantly greater in the RPA group than in the control group (P < 0.0001). * Mann–Whitney U Test.

Values are shown as means ± SD.

**Figure 1** — Paraoxonase levels according to group.
Discussion

Regular physical activity is associated with a reduced risk of cardiovascular disease (16), and consequently physical inactivity is considered a risk factor for cardiovascular disease (30). To our knowledge, the protective role of RPA in paraoxonase activity and oxidative status, specifically the effects of such activity on juvenile amateur athletes, have not yet been established. Many studies have indicated, however, that paraoxonase and arylesterase may serve to help prevent the development of atherosclerosis (56,29).

Studies in animal models, including paraoxonase-knockout mice, have indicated that paraoxonase deficiency is related to increased susceptibility to LDL oxidation and atherosclerosis development (48). In humans, it has been suggested that paraoxonase is significantly associated with CHD risk (30). There is accumulating evidence that reduced activity of the HDL-associated (5) enzyme paraoxonase is predictive of vascular disease in humans, including results obtained in prospective studies (23,40). The role of paraoxonase in vascular disease was also strongly supported by the results of knockout and transgenic mouse studies. Knockout mice lacking serum paraoxonase show increased susceptibility to

Table 2  Oxidative and Antioxidative Parameters of RPA and Control Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RPA groups (n = 64)</th>
<th>Control group (n = 32)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraoxonase (U/L)</td>
<td>177.32 ± 100.10</td>
<td>98.11 ± 40.92</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Arylesterase (U/L)</td>
<td>98.66 ± 50.05</td>
<td>55.05 ± 20.46</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>TAC (mmol Trolox equiv/L)</td>
<td>1.07 ± 0.14</td>
<td>0.92 ± 0.12</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>LOOH (mmol H2O2 equiv/L)</td>
<td>3.95 ± 0.9</td>
<td>3.05 ± 0.43</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>TOS (mmol H2O2/L)</td>
<td>11.15 ± 3.20</td>
<td>8.11 ± 2.27</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>OSI (AU)</td>
<td>10.67 ± 3.63</td>
<td>9.0 ± 2.93</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Note. Values are expressed as means ± SD

Table 3  Lipid Profiles of the RPA and Control Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RPA groups (n = 64)</th>
<th>Control group (n = 32)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.47 ± 0.87</td>
<td>1.66 ± 1.29</td>
<td>0.39</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.62 ± 1.06</td>
<td>4.54 ± 1.01</td>
<td>0.22</td>
</tr>
<tr>
<td>HDL-Cholesterol (mmol/L)</td>
<td>1.37 ± 0.34</td>
<td>1.39 ± 0.39</td>
<td>0.65</td>
</tr>
<tr>
<td>LDL-Cholesterol (mmol/L)</td>
<td>2.5 ± 0.58</td>
<td>2.37 ± 0.71</td>
<td>0.55</td>
</tr>
<tr>
<td>VLDL-Cholesterol (mmol/L)</td>
<td>0.67 ± 0.40</td>
<td>0.76 ± 0.59</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Note. Values are expressed as means ± SD
atherosclerosis (7), with a 42% increase in rate of stenosis compared with controls (42). Conversely, human serum paraoxonase transgenic mice with 2- to fourfold increased paraoxonase levels show reduced susceptibility (53). A previous study indicated that the activity of paraoxonase is associated with age and HDL (26). In another study, the activity of paraoxonase was shown to be negatively correlated with age and positively correlated with HDL (22). In a population-based study, paraoxonase activity levels were significantly higher in physically active subjects than in those who were inactive (46). A comparison of paraoxonase activity between groups matched for age and gender indicated that paraoxonase activity was significantly higher in the study group than in the controls. A significant increase in paraoxonase was reported in a study on adults undertaking a 12-week exercise program (17).

All oxidant antioxidant parameters (TOS, OSI, LOOH, TAC) in the RPA group were higher than those in the control group in the current study. Although aerobic training increases antioxidant activity in animal models (37), the effects of aerobic training on antioxidant activity in humans are controversial. Higher levels of antioxidant enzyme activity have been observed in physically active subjects than in sedentary subjects (11). Some studies have indicated an increase in antioxidant enzyme activity after training (58), whereas others have documented no changes (51) or even a decrease (4) in circulating antioxidants. In the current study, TAC levels were greater in the athletic subjects. This increase in activity may have been due to endogenous antioxidant production related to the repeated increases in levels of free radicals acting as inductors of gene transcription after each PA session (45). Various other studies have shown that the antioxidant capacity increases as oxidative stress increases as a protective mechanism (25). Thus, there is increased vulnerability to long-term oxidative stress and so the development of the antioxidant system could occur as a response. In humans, regular exercise appears to enhance antioxidant systems and improve plasma oxidant status (21). The total antioxidant activity, enhanced by physical exercise, may indirectly mitigate the inhibition of paraoxonase caused by acute exercise-induced oxidative stress, and therefore paraoxonase activity may recover to basal levels faster in trained individuals. In addition to these effects, exercise may also exert a direct action on the paraoxonase protein or paraoxonase carrier lipoprotein (59).

Paraoxonase is mainly associated with HDL particles (8). However, no correlation was found between HDL-C levels and paraoxonase activity in any subject in the current study. Therefore, it is unlikely that HDL-C differences can explain the observed differences in paraoxonase activity. Other studies (17,59,52) indicated no significant differences in lipid levels between the study and control groups, and the findings of the current study were consistent with these observations.

Studies evaluating the correlation between paraoxonase activity and lipids have indicated no correlation between the study and control groups (59,52). In agreement with these previous reports, we found no significant correlation between paraoxonase activity and lipid profiles in the current study. In previous studies, a negative correlation was reported between paraoxonase activity and oxidative status, and a positive correlation was found with antioxidants (43,44). In the current study, however, no significant correlations were found between paraoxonase / arylesterase and TAC, TOS, LOOH, or OSI in the study group or in the controls. Regardless of whether there are positive or negative correlations between paraoxonase
Nutrition can yield important changes in paraoxonase activity. An atherogenic diet reduces paraoxonase activity in mice, and this decrease is correlated with a decrease in plasma HDL-cholesterol (48). Pomegranate juice consumption (18) or the intake of vitamin C and E supplements (24) may increase serum paraoxonase activity. Ethanol has been shown to inhibit serum paraoxonase activity (12), but moderate alcohol ingestion seems to have the opposite effect. These effects are probably secondary to the increase in HDL concentration (54). Subjects in neither the study group nor the control group were following any special diet, and there were no differences between the two groups in nutrition or calorie intake. Whether nutrition had any effect on paraoxonase activity in the study and control groups was not assessed as a parameter in the current study.

The present study had potential limitations in that we did not analyze any of the paraoxonase polymorphisms in the 5¢ promoter region. However, several paraoxonase gene promoter polymorphisms have been described recently that influence the enzyme’s serum concentration (3).

In conclusion, the protective impact of RPA in the development of atherogenesis is well known. The increases in the activities of paraoxonase and arylesterase, enzymes with antioxidant and antiatherosclerotic properties, in RPA may influence both the progression of the disease and the development of atherosclerosis. Further studies are needed to clarify the possible mechanisms underlying the increases in enzyme activities.

**References**


