Exercise Increases the Plasma Antioxidant Capacity of Adolescent Athletes

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Key Words
Oxidative stress • Exercise-induced adaptations • Competitive athletes • Antioxidants

Abstract

Background: The reactive oxygen species produced as a result of exercise might exceed an individual's antioxidant defence system. Various endogenous antioxidants are elevated in adult athletes, resulting in an improved antioxidant capacity. However, little is known about antioxidant defence in adolescents. The purpose of this study was to examine presumed adaptations of antioxidant capacity in exercising adolescents. Methods: Trolox-equivalent antioxidant capacity (TEAC), uric acid and nutritional antioxidants were measured in the plasma of 91 male and 98 female athletes (mean age 15.9 ± 2.0 years) and compared to those of 18 male and 22 female sedentary controls (mean age 16.3 ± 2.1 years). Antioxidant intake was calculated using 4-day dietary records. Results: Neither male nor female athletes showed differences in α-tocopherol, β-carotene or ascorbate intake compared to controls. Plasma levels of α-tocopherol and carotenoids in athletes and controls did not differ either. Nevertheless, athletes of both sexes had higher TEAC values than their respective controls (male athletes 1.48 ± 0.22 mmol/l vs. male controls 1.23 ± 0.19 mmol/l, female athletes 1.47 ± 0.20 mmol/l vs. female controls 1.15 ± 0.04 mmol/l, p < 0.05). Conclusions: Regular exercise enhances antioxidant capacity in adolescent athletes, independently of their dietary antioxidant intake, which indicates activity-related adaptations.

Introduction

Reactive oxygen species (ROS) are produced within the body as a result of various physiological and pathological conditions. Because of their unpaired electrons, ROS are highly reactive and may destroy macromolecules, cells and tissues. Oxidative stress is known to be a risk factor for a variety of diseases, and an imbalance in redox state has been implicated in the development of muscle disturbances such as chronic fatigue syndrome or delayed onset of muscle soreness [1]. However, recent research suggests that ROS produced in contracting skeletal muscle play a pivotal role in adaptation to training, as they may activate redox-sensitive signalling pathways, which stimulate growth, differentiation, proliferation or apoptosis [2, 3]. There is a rising body of evidence that the
cellular redox state influences the contractile muscle function as redox-sensitive proteins are responsible for coupling the excitation and metabolic pathways [4, 5]. A certain ROS concentration seems to be necessary to maintain normal muscle force and contractility [4, 6]. However, the regulation of ROS concentration within narrow physiological limits might be an essential mechanism to assure cell function and prevent oxidative damage or muscular fatigue [3, 7].

Exercise has been associated with increased ROS formation as a result of elevated oxygen consumption [8, 9]. During exhaustive exercise, whole body oxygen consumption increases by 15- to 20-fold, while in the working muscles values of 100- to 200-fold above resting levels may be reached [10]. Exercise was subsequently believed to enhance ROS production because of activation of the mitochondrial respiratory chain [11]. However, there is a substantial body of evidence that sources other than mitochondrial leakage contribute considerably to exercise-induced ROS generation [12]. During strenuous muscular work, the calcium-dependent xanthine oxidase pathway plays a pivotal role in superoxide formation [8]. Damaging exercise often entails an immune response [13] and contraction-induced myofibrillar disruptions result in the activation of various immune-responsive cells. Neutrophils migrating into the damaged muscle might further enhance ROS production as a result of their oxidative bursts [14]. Recent research indicates that ROS formation by membrane-bound oxidoreductases, such as NAD(P)H oxidase, is elevated during the contractile activity of skeletal muscle [15].

Although there is evidence that moderate physical activity might disrupt the redox balance [8], exercise-induced adaptations of antioxidant capacity in adults have been shown by numerous investigators. These adaptations include altered activities and gene expressions of antioxidant enzymes such as superoxide dismutase, catalase or glutathione peroxidase [16, 17]. An insufficient antioxidant defence system may allow the development of oxidative stress, which is associated with metabolic alterations that lead to poorer physical performance in athletes [18]. Athletes are at particular risk of exceeding their antioxidant capacity when they train too intensely or perform exercises to which they are not accustomed. This is accompanied by increased lipid peroxidation and muscle damage [19, 20]. However, there are conflicting results concerning the effects of nutritional antioxidants or supplements on the prevention of post-exercise oxidative stress [21, 22]. Moreover, antioxidant supplementation might impair ROS formation as the underlying mechanism for training adaptation and elevated expression of antioxidant enzymes [2, 5].

There is little information available regarding exercise-induced adaptations of the ROS defence system in adolescent and child athletes. Precise knowledge about the assumed training-induced up-regulation of endogenous antioxidants in exercising children could be helpful in preventing overtraining, fatigue and muscular damage in teenage athletes. The main objective of this study was to determine whether exercise-dependent alterations within the antioxidant defence capacity are observed in children and adolescents exercising regularly. Furthermore, we addressed the question whether the type of exercise, age, gender or the number of weekly training sessions influence the antioxidant capacity of teenage athletes.

Subjects and Methods

Subjects, Physical Activity and Dietary Intake

The study was approved by the Ethics Committee of the University of Potsdam and the Brandenburg Ministry of Education, Youth and Sports. Written informed consent was given by all subjects, and their parents in cases where participants were underage. Volunteers were recruited either from students at a school for elite sports (athletes, n = 189) or from a school with no emphasis on physical activity (sedentary controls, n = 40). The mean age of participants was 15.9 ± 2.0 years for athletes and 16.3 ± 2.1 years for controls. Athletes represented 14 different disciplines, and were placed into the following groups according to their main exercise emphasis: (1) endurance athletes (n = 84), (2) quick-strength athletes (n = 48), (3) athletes involved in ballgames (n = 41), and (4) alumni athletes (n = 16) having retired from a competitive career but still engaging in moderate physical activity. Physical activity was evaluated on the basis of self-report of the number of training sessions per week. Volunteers were divided into the following 4 activity levels: (1) non-active (0–2 h of sports/week), (2) moderately active (2–6 h of sports/week), (3) middle-range-performance athletes (7–12 training sessions/week) and (4) high-performance athletes (>12 training sessions/week).

Individual supply of energy, macro- and micronutrients was assessed using a 4-day dietary protocol, which discriminated between cooked and uncooked fruits and vegetables [23]. Nutritional antioxidant intake by habitual diet was calculated based on the German Food Code and Nutrition Database BLS II.3, additional supply was quantified by a supplement questionnaire.

Blood Sample Collection and Anthropometric Measurements

Blood sample collection was performed after overnight fasting and before the first training session of the day. Blood was drawn from an antecubital vein into pre-chilled test tubes with EDTA as anticoagulant (Kabevette E301, Kabe, Nümbrrecht-Elsenroth, Germany). Plasma was obtained by centrifugation for 15 min at 2,500 g, and analysis aliquots were stored at –80°C. Height, weight and body composition were measured in the morning as fasting
values. Percentage body fat was determined using the BIA 2000-M multi-frequency body-composition analyzer (Data Input, Frankfurt-Main, Germany).

**Analysis of α-Tocopherol, Carotenoids, Uric Acid and Total Antioxidant Capacity**

Plasma levels of carotenoids and α-tocopherol were analyzed within a month after blood sample collection. For separation and quantification of α-tocopherol and carotenoids (lutein, zeaxanthin, α- and β-carotene, β-cryptoxanthin and lycopene) a modified reverse-phased HPLC system was used, as previously described [24]. Uric acid quantification was carried out on Olympus AU 600 using the Uricase-PAP test (Olympus Europe GmbH, Hamburg, Germany). Plasma antioxidant capacity was determined by a modified Trolox-equivalent antioxidant capacity assay and expressed as Trolox equivalents (TE) as described elsewhere [25].

**Statistical Analysis**

The results are expressed as means ± standard deviations. All statistical calculations were carried out on SPSS 14.0 for Windows. The unpaired Student t test (athletes vs. controls) at 95% confidence interval and Kruskall-Wallis test were used to assess differences between the groups. Correlations were analysed using Pearson’s correlation coefficient.

**Results**

**Subject Characteristics, Physical Activity and Macronutrient Intake**

There were no significant differences in age, BMI or percentage body fat between athletes and sedentary controls. Detailed characteristics are listed in table 1. As expected, most of the athletes fell into ‘mid-range performance’ or ‘high performance’ categories (fig. 1). For males, energy and macronutrient intake was comparable between the different discipline groups, alumni athletes and sedentary controls. Among the athletes, some discipline-specific variations in energy intake and proportion of macronutrients were found. Except for alumni athletes, females had similar energy and macronutrient supply in their diets. Alumni female athletes consumed significantly less carbohydrate, protein and fat (all p < 0.05), resulting in a lower total energy intake (table 2).

**Dietary Antioxidant Intake**

The habitual diets of the males involved in various sports did not differ in their nutritional antioxidant content (table 2). The low intake of foods rich in energy and fat by female alumni athletes was associated with a decreased consumption of dietary antioxidants (table 2). Increase in total energy intake was accompanied by improved supply of β-carotene and vitamins C and E (table 3). Non-dietary antioxidant intake by supplements was negligible in both athletes and controls.

**Plasma Content of Nutritional Antioxidants**

There were no differences in plasma carotenoid and α-tocopherol levels in athletes versus sedentary controls. Among the athletes, male high-performance athletes had higher β-carotene, total lycopene and total carotenoid levels compared to moderately active students (p < 0.05).
Table 2. Macronutrient intake and supply with nutritional antioxidants within the various discipline groups

<table>
<thead>
<tr>
<th>Sports type</th>
<th>Energy MJ/day</th>
<th>Carbohydrate g/day</th>
<th>Protein g/day</th>
<th>Fat g/day</th>
<th>β-Carotene mg/day</th>
<th>Vitamin E mg/day</th>
<th>Vitamin C mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endurance</td>
<td>12.9 ± 4.8a</td>
<td>397 ± 146</td>
<td>125 ± 55a</td>
<td>105 ± 45a</td>
<td>5.5 ± 3.9</td>
<td>21.1 ± 10.7</td>
<td>251 ± 180</td>
</tr>
<tr>
<td>Quick strength</td>
<td>10.4 ± 1.7a</td>
<td>353 ± 58</td>
<td>98 ± 20</td>
<td>71 ± 20a</td>
<td>4.4 ± 2.0</td>
<td>21.3 ± 10.2</td>
<td>228 ± 79</td>
</tr>
<tr>
<td>Ballgames</td>
<td>13.4 ± 5.7</td>
<td>424 ± 165</td>
<td>120 ± 58</td>
<td>108 ± 54</td>
<td>3.0 ± 2.4</td>
<td>33.3 ± 21.2</td>
<td>238 ± 143</td>
</tr>
<tr>
<td>Alumni</td>
<td>11.4 ± 1.9</td>
<td>390 ± 95</td>
<td>101 ± 17</td>
<td>75 ± 11b</td>
<td>3.4 ± 2.2</td>
<td>15.7 ± 6.1</td>
<td>159 ± 82</td>
</tr>
<tr>
<td>Controls</td>
<td>10.7 ± 3.9</td>
<td>335 ± 120</td>
<td>91 ± 32a</td>
<td>90 ± 38</td>
<td>4.8 ± 2.6</td>
<td>15.7 ± 7.1</td>
<td>185 ± 115</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endurance</td>
<td>9.0 ± 2.1</td>
<td>283 ± 76</td>
<td>86 ± 21</td>
<td>70 ± 24</td>
<td>4.9 ± 3.1</td>
<td>18.8 ± 12.3</td>
<td>197 ± 124</td>
</tr>
<tr>
<td>Quick strength</td>
<td>9.4 ± 2.9</td>
<td>306 ± 95</td>
<td>82 ± 27</td>
<td>72 ± 26</td>
<td>3.9 ± 3.1</td>
<td>16.9 ± 9.3</td>
<td>186 ± 108</td>
</tr>
<tr>
<td>Ballgames</td>
<td>9.7 ± 2.7</td>
<td>310 ± 101</td>
<td>93 ± 27</td>
<td>73 ± 24</td>
<td>4.3 ± 3.4</td>
<td>17.2 ± 7.2</td>
<td>172 ± 106</td>
</tr>
<tr>
<td>Alumni</td>
<td>6.5 ± 1.5*</td>
<td>217 ± 72*</td>
<td>60 ± 15*</td>
<td>46 ± 8*</td>
<td>2.8 ± 1.5</td>
<td>8.9 ± 2.9*</td>
<td>102 ± 60</td>
</tr>
<tr>
<td>Controls</td>
<td>8.9 ± 2.5</td>
<td>281 ± 75</td>
<td>78 ± 28</td>
<td>73 ± 24</td>
<td>4.9 ± 3.8</td>
<td>15.2 ± 5.7</td>
<td>164 ± 89</td>
</tr>
</tbody>
</table>

Data are means ± SD. The same superscript letters in one column indicate significant intra-gender differences (p < 0.05).
* Female alumni athletes showed significant lower intake of energy, carbohydrates, protein, fat and vitamin E compared to all other groups, except for carbohydrates and protein compared to sedentary controls.

Table 3. Intake and plasma levels of dietary antioxidants and plasma antioxidant capacity (TEAC) depending on physical activity

<table>
<thead>
<tr>
<th>Activity level</th>
<th>Dietary intake</th>
<th>Plasma levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>energy MJ/day</td>
<td>β-carotene μmol/l</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderately active</td>
<td>10.6 ± 2.7a</td>
<td>3.8 ± 2.3</td>
</tr>
<tr>
<td>Mid-range performance</td>
<td>11.9 ± 4.7b</td>
<td>5.0 ± 3.8</td>
</tr>
<tr>
<td>High performance</td>
<td>15.1 ± 3.9b</td>
<td>4.9 ± 3.1</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-active</td>
<td>8.8 ± 2.6d</td>
<td>4.1 ± 2.2</td>
</tr>
<tr>
<td>Moderately active</td>
<td>7.6 ± 2.5c,d</td>
<td>4.0 ± 4.0</td>
</tr>
<tr>
<td>Mid-range performance</td>
<td>9.3 ± 2.6c</td>
<td>4.3 ± 3.1</td>
</tr>
<tr>
<td>High performance</td>
<td>10.4 ± 1.6d</td>
<td>5.6 ± 3.6</td>
</tr>
</tbody>
</table>

Data are means ± SD. The same superscript letters in one column indicate significant differences (p < 0.05).
1 Total lycopene is the sum of cis- and trans-lycopene.
2 Total carotenoids are the sum of lutein, zeaxanthin, α- and β-carotene, β-cryptoxanthin and lycopene.
3 Only one male non-active volunteer gave in a dietary report.

This was not observed in females, except for the higher α-tocopherol levels in the high-performance athletes compared to moderately active females (p < 0.05, table 3). We observed no correlation between TEAC values and elevated plasma levels of α-tocopherol or carotenoids.

**Plasma Antioxidant Capacity (TEAC)**

Athletes and those having abandoned their competitive career (alumni) showed significantly (p < 0.05) elevated plasma antioxidant capacity in both males (1.48 ± 0.22 mmol TE/l) and females (1.47 ± 0.20 mmol TE/l) compared to sedentary controls (1.23 ± 0.19 mmol TE/l for males and 1.15 ± 0.04 mmol TE/l for females; fig. 2). No differences in TEAC values were observed between endurance athletes, quick-strength athletes and ballgame players (fig. 3). There was no evidence for gender-dependent variations in antioxidant capacity (table 3). In both male (Pearson’s correlation r = 0.47, p < 0.05) and female
athletes ($r = 0.63$, $p < 0.05$) the antioxidant capacity increased with rising age. However, this apparently age-dependent elevation of plasma antioxidant capacity was not seen in non-athletes (fig. 4). There were no differences observed for uric acid between athletes and controls (male athletes $333 \pm 125$ µmol/l vs. male controls $315 \pm 65$ µmol/l, female athletes $232 \pm 42$ µmol/l vs. female controls $243 \pm 42$ µmol/l).

Finally, a significant correlation between the number of training sessions per week and antioxidant capacity was obvious ($r = 0.41$, $p < 0.05$). The scale of antioxidant plasma capacity ranged from $1.13 \pm 0.03$ mmol TE/l in the non-active to $1.51 \pm 0.23$ mmol TE/l in the high-performance athletes (fig. 5). This finding turned out to be significant for both the sexes as well as for athletes and non-athletes alike (table 3).
Discussion

It is generally accepted that plasma antioxidant capacity does not reflect the cellular redox state. However, with the limitations of measuring total plasma antioxidant capacity in mind, we applied the TEAC assay to examine whether and to what extent the antioxidant capacity of children and adolescents is adapted to their ambitious training regime [26]. The TEAC assay has been shown to give reliable data for assessing antioxidant response to exercise [27], disease [28] or dietary intervention [29] in different human body fluids. Athletes and non-athletes showed similar characteristics according to age, anthropometric data, energy and macronutrient intake, which indicates TEAC modulations independent of diet. In athletes, but not in sedentary controls, antioxidant capacity increased remarkably with rising age. Thus, we suppose the putative age dependency of antioxidant capacity was not only due to maturation, but rather was a result of an age-associated component. The training regime might be such an age-associated factor that can influence the antioxidant defence system. In this respect, the duration, intensity and number of anaerobic-lactacid training sessions per week is assumed to increase with rising age in young athletes. The hypothesis of an age-independent rise in plasma TEAC is supported by our finding that these values increase continuously in athletes with the number of weekly exercises. High-performance and mid-range-performance athletes have significantly elevated antioxidant capacities compared with non-active and moderately active students. The raise in antioxidant capacity with increasing training effort suggests a dose-dependent mechanism of adaptations to exercise-induced increase in ROS formation.

Plasma carotenoids were similar to other study cohorts of comparable age and reflect a sufficient intake of fruits and vegetables [30]. Although TEAC values are primarily influenced by diverse water-soluble substances, we measured fat-soluble plasma antioxidants such as carotenoids and tocopherols. A lack of difference in plasma carotenoid content reflects a similar intake of plant foods and plant-derived antioxidants in athletes and controls, as it is well documented that plasma carotenoids serve as biomarkers for the habitual consumption of fruits and vegetables [31]. All groups met the daily recommendations for the intake of vitamins C and E. Among our well-nourished study population that was homogenous according to nutritional antioxidant intake, we could not find any correlation between TEAC and α-tocopherol, ascorbic acid and β-carotene consumption, or with plasma levels of α-tocopherol and carotenoids. The absence of differences in the dietary antioxidant intake between athletes and controls suggests a nutrition-independent, exercise-associated TEAC elevation. In conclusion, we propose that exercising itself might influence TEAC values in the plasma of adolescent athletes, as has been shown in adults [32, 33]. However, assumed adaptations need to be studied in untrained adolescents using training schedules to evaluate response to standardized exercises.

The data presented here do not support the idea of gender differences in the antioxidant defence systems in athletes, which is in agreement with a recently published report [34]. Although both sedentary and exercising females have lower plasma TEAC levels, this finding did not reach statistical significance, and in a detailed analysis conflicting results within the different discipline groups were observed. This outcome is in contrast to the thesis that females might have a higher antioxidant potential due to the antioxidant effect of sex hormones such as 17β-estradiol [35]. Katalinic et al. [36] observed a higher antioxidant tissue capacity and lower susceptibility to oxidative modulations in female rats. In humans, postmenopausal women seem to be more susceptible to oxidative stress than premenopausal women, indicating a relationship between oestrogens and antioxidant capacity [37]. Conflicting statements concerning the influence of sex hormones on the female redox balance might be due to the potential of oestrogens to act both as antioxidants and pro-oxidants [38]. Moreover, the possibility of a decreased oestrogen blood concentration in female athletes due to exercise-induced hormonal misbalances must be taken into consideration [39].

When comparing antioxidant capacity of athletes in different sports, no difference could be revealed. Although different sports are known to affect ROS formation unequally, athletes had almost identical mean TEAC values, independently of the chosen discipline. This finding might be due to the fact that young athletes in all sports complete a high proportion of non-specific fundamental training that includes similar elements. Almost all the above-mentioned disciplines require components of both aerobic and anaerobic endurance, speed, quick strength, strength endurance and strength in general. Thus, having studied the athletes during a semi-specific period of training accentuation in their yearly cycle, no discipline-specific variances could be anticipated. In addition, we cannot provide any evidence-based explanation for the significant TEAC elevation in alumni athletes compared to active athletes and controls. However, as
those athletes have been retiring from competitive career no longer than 1 year prior to blood sample collection, a lag phase before reversal of adaptation can be anticipated. No data are available about existence and duration of this assumed lag phase. Moreover, alumni athletes involved in the study still showed above-average physical activity, which might have been sufficient to inhibit reversal of adaptation, keeping antioxidant capacity at a high level. Yet already one single intensive exercise programme is known to reduced GSH levels or decrease the GSH-to-GSSG ratio [40]. An increased enzymatic antioxidant defence concomitant with a lower reduction of GSH-to-GSSG ratio resulting from lower exercise intensity and frequency might result in the higher overall antioxidant capacity of alumni athletes compared to active competitors.

It is not possible to say with certainty which endogenous antioxidant modifications are responsible for the raise in TEAC values in our subjects. Calculating the mean serum concentrations and measuring the antioxidant capacity of the following substances, Miller et al. [41] suggested that albumin (43%), uric acid (33%) and vitamin E (3%) might be the most relevant antioxidants to influence serum TEAC values. However, in another study their contribution was estimated to be lower, at 28% for albumin, 19% for uric acid and 2% for α-tocopherol [42]. As production of uric acid is increased during exercise due to increased AMP breakdown, it could be assumed that the observed modulation of plasma antioxidant capacity is primarily based on increased uric acid levels. However, significant differences in plasma uric acid content between athletes and controls are lacking. Thus, we suggest that antioxidants other than uric acid might be modified. There is evidence that exercise-induced TEAC elevation might be caused by induction of plasmatic enzymes, as has been shown for superoxide dismutase [32, 43, 44], glutathione reductase [45] and low molecular weight compounds such as plasma thiols [46].

**Conclusion**

Our results indicate that regular physical activity evokes adaptations in the antioxidant defence system that appear to be independent of diet and age. Further research is required, including investigations into enzyme activities, glutathione content and the behaviour of other endogenous antioxidants to analyse the mechanisms involved in the plasma antioxidant defence system. The evaluation of oxidative modulations is necessary to answer the question whether the above-demonstrated adaptations of the antioxidant defence system are sufficient to protect adolescent athletes who are highly exposed to ROS from subsequent oxidative damage.

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