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Effect of Astaxanthin Supplementation on Paraoxonase 1 Activities and Oxidative Stress Status in Young Soccer Players

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The purpose of the study was to examine the effects of astaxanthin (Asx) on paraoxonase (PON1) activities and oxidative stress status in soccer players. Forty soccer players were randomly assigned in a double-blind fashion to Asx and placebo (P) group. Blood samples were obtained before, 45 and 90 days after supplementation. PON1 activity was assessed by using two substrates: paraoxon and diazoxon. The oxidative stress biomarkers were also examined: total sulphydryl group content (–SH groups), thiobarbituric acid-reactive substances (TBARS), advanced oxidation protein products and redox balance. The significant interaction effect of supplementation and training ($p < 0.05$) on PON1 activity toward paraoxon was observed. The PON1 activity toward diazoxon increased in Asx group after 90 days ($p < 0.01$), while there was no significant difference in P group. SH groups content rose from pre- to post-supplementation period only in Asx group (supplementation and training, $p < 0.05$; training, $p < 0.01$). TBARS levels decreased after 45 days and increased after 90 days of regular soccer training in both groups (training, $p < 0.001$). Redox balance decreased significantly in response to the regular training, regardless of treatment group (training, $p < 0.001$). Asx supplementation might increase total SH groups content and improve PON1 activity through protection of free thiol groups against oxidative modification.

Keywords: astaxanthin; soccer; paraoxonase 1 activity; oxidative stress.

INTRODUCTION

Intense physical activity is accompanied by an increased generation of reactive oxygen species (ROS), resulting in a measurable degree of oxidative modifications to various molecules (Vollaard *et al*., 2005). Despite regular exercise having a protective effect through improvement of antioxidant capacity (Brites *et al*., 2000), it is likely that intense training sessions expose participants to oxidative stress and damage both during and, immediately post-exercise, as well as throughout the recovery phase (Ascensao *et al*., 2008). Moreover, an athlete's antioxidant dietary intake is frequently inadequate, which may increase their susceptibility to exercise-induced oxidative damage (Margaritis *et al*., 2003). For these reasons, supplementation with exogenous antioxidants may provide support for the endogenous antioxidant defense system in order to assist in the handling of the increased production of ROS.

Paraoxonase 1 (EC 3.1.8.1, aryldialkylphosphatase, PON1) is an antioxidant enzyme synthesized in the liver and secreted into the blood where it mainly binds to high density lipoprotein (HDL) (Draganov and La Du, 2004). PON1 has an inhibitory effect on the formation of atherosclerotic plaques, as PON1 is able to protect low density lipoproteins (LDL) and HDL against oxidative modification by ROS (Costa *et al*., 2011). In addition, it seems that PON1 has several other roles and may have a number of various biological substrates. However, in the absence of an acknowledged biological substrate for PON1, its activity can be determined through its degrading function towards artificial substrates, such as paraoxon, diazoxon and phenyl acetate (Otocka-Kmiecik and Orłowska-Majdak, 2009). PON1 activity is modulated at the PON1 locus, by the $PON1_{192}$ and $PON1_{55}$ polymorphisms, among others. The $PON1_{192}$ polymorphisms comprise Q and R alleles, whose protein allozymes have been suggested to differ both in the PON1 activity and in their ability to confer protection against oxidative conditions (Q allele for lower activity toward paraoxon and higher protection than R allele) (Costa *et al*., 2011). However, environmental factors, such as diet, lifestyle and pharmacologic therapy may modulate its activity (Ferre *et al*., 2003). Given the role of PON1 in protecting against cardiovascular disease and its decrease in a number of pathological conditions (Costa *et al*., 2011), it is not surprising that particular attention has been devoted to factors that may modulate PON1 activity.

Among the many recognized factors accounting for lifestyle, physical activity plays an important role. Several studies have showed that regular physical activity might increase PON1 activity. Senti *et al*. showed that physically active subjects had increased PON1 levels,

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measured as paraoxonase activity, compared to a sedentary population (Senti *et al*., 2003). Arslan *et al*. observed marked increase in PON1 activity toward paraoxon with longer training experience and an increased number of exercise sessions per week (Arslan *et al*., 2005). However, some studies failed to show an increase in PON1 activity in subjects who practiced regular physical activity (Richter *et al*., 2005; Brites *et al*., 2006). In addition, regular training in a 6 week period did not affect PON1 activity in women volleyball players (Martinovic *et al*., 2011). Also, neither vigorous nor moderate aerobic exercise after 8 weeks had significant effects on PON1 activity in non-active healthy men (Gharakhanlou *et al*., 2007).

As PON1 is easily inactivated by ROS (Nguyen and Sok, 2003), several strategies to increase PON1 have focused on the administration of dietary antioxidants. Application of vitamin C and E, pomegranate juice, quercetin or resveratrol seems promising since the majority of the studies have showed that the activity of PON1 can be significantly increased as a consequence of dietary supplementation (Aviram *et al*., 2004; Boesch-Saadatmandi *et al*., 2010; Jarvik *et al*., 2002).

Astaxanthin (3,3′-dihydroxy-β,β′-carotene-4,4′-dione; Asx) is one of the main pigments belonging to the family of the xanthophylls, which is generally distributed in seafood, for example, in salmon, prawn and crab. Asx has been shown to be one of the most effective antioxidant against lipid peroxidation and oxidative stress in *in vitro* and *in vivo* systems (Tripathi and Jena, 2009; Chan *et al*., 2009; Choi *et al*., 2011). Growing evidence suggests that Asx has potential health-promoting effects in the prevention and treatment of various diseases (cancers, chronic inflammatory diseases, diabetes, cardiovascular disease, neurodegenerative diseases) (Yuan *et al*., 2011). Most research on the actual benefits of Asx as a dietary supplement has been limited to *in vitro* assays and animal studies. Thus, a prospective, randomized, double-blind, placebo controlled trial was conducted to determine the effects of Asx supplementation on PON1 activity and oxidative stress status in young soccer players while following their habitual dietary patterns and regular training program during a competitive season.

MATERIAL AND METHODS

Subjects. Forty male soccer players, recruited from the junior selection of soccer club 'Partizan' in Belgrade, Serbia, participated in this 12-week double blind study. All participants were healthy, non-smokers, had no ongoing or previous (during last year) injuries and reported no use of any medication or antioxidant supplements for at least 3 months. Subjects were instructed to refrain from making any drastic changes in their diet and also to abstain from anti-inflammatory, analgesic drugs or supplements throughout the study.

This study was conducted according to the guidelines laid down in Declaration of Helsinki. All procedures were approved by the Ethical Committee of the Sports Medicine Association of Serbia. Athletes (or their parents) gave written informed consent after verbal and written explanation of the purpose, demands and possible risks associated with the study.

Study design. Subjects were randomly assigned in a double-blind fashion to one of two treatment groups. The Asx group ($n = 21$) was supplemented with 4 mg of Asx (natural Asx derived from microalgae *Haematococcus pluvialis*) for 90 successive days. The placebo (P) group ($n = 19$) was given capsules, identical in appearance and taste, containing saccharose.

During the first 8 weeks of the observational period, the players performed 5 weekly training sessions (~525 min) on the football field, in addition to a weekly match. During the subsequent 5 weeks, they had 6 weekly training sessions (~670 min) in addition to a weekly match. The training session consisted of a continuous running series of low to moderate intensity, intervallic training of moderate to high intensity, physical-technical circuits and game-like activities with small groups and large spaces. This training was complemented by two weight sessions per week (~115 min).

Prior to enrollment into the study, all subjects completed a maximal oxygen consumption test (VO_{2max}), body composition assessment, 4-day diet record and general health-screening questionnaire. VO_{2max} was measured on a motor-driven treadmill (Run race, Techno gym, Italy), using an indirect calorimetry system (Quark b2, Cosmed, Italy) with an incremental exercise test to volitional fatigue. Height was measured to the nearest 0.1 cm with a portable stadiometer. Body mass and total body fat were measured using the BC-418, eight-contact electrode device (Tanita, Tokyo, Japan), which uses bioelectrical impedance analysis for body composition analysis. Body mass index was calculated by dividing the weight in kilograms by the square of the height in meters (kg/m^2). The energy, macronutrient and micronutrient intakes were calculated using CRON-O-Meter v0.9.6. software.

Laboratory investigations. Blood sample collection was performed between 9:00 and 10:00 after a 10 h overnight fast, at three time points: before supplementation (baseline), after 45 days of supplementation (45 day) and after 90 days of supplementation (90 day). Venous blood was collected into a sample tube with serum separator gel (for serum) and into a heparin evacuated tube (for plasma), (Greiner Bio-one, Kremsmünster, Austria). Blood samples were transported and stored in the laboratory where analyses were performed strictly following international guidelines (Banfi and Dolci, 2003). Serum and plasma were separated by centrifugation at $1500 \times g$ for 10 min, and multiple aliquots of each sample were stored at $-80\,^{\circ}C$ until analysis.

Total cholesterol (CHOL), HDL cholesterol (HDL-C) and triglycerides (TG) were measured in serum using an ILab 300 Plus autoanalyzer employing commercial kits (Bioanalytica, Belgrade, Serbia). The concentration of LDL cholesterol (LDL-C) was calculated using the Friedewald formula (Friedewald *et al*., 1972).

PON1 activity was assessed spectrophotometrically using two different substrates: paraoxon (paraoxonase activity-POase) and diazoxon (diazoxonase activity-DZOase). The conversion of paraoxon to *p*-nitrophenol by the hydrolytic activity of PON1 was monitored at 405 nm. PON1 activity toward diazoxon was determined by the rate of diazoxon hydrolysis and the subsequent production of 2-isopropyl-4-methyl-6-hydroxypyrimidine, monitored at 270 nm (Richter and Furlong, 1999). The activities are reported as μmol/min/L (noted as IU/L).

By measuring the DZOase/POase activity ratio, we discriminated $PON1_{Q192}$, $PON1_{QR192}$ and $PON1_{R192}$ phenotypes.

The concentration of sulphydryl groups (–SH, mmol/L) was determined by using 0.2 mmol/L 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB). SH groups react with DTNB, cleaving the disulfide bond to give 2-nitro-5-thiobenzoate (NTB^-), which ionizes to the NTB^{2-} dianion in water at neutral and alkaline pH. The NTB^{2-} ion was quantified spectrophotometrically at 412 nm (Ellman, 1959). The concentration of thiobarbituric acid-reactive substances (TBARS) was measured by using the TBARS assay employing the molar absorption coefficient of $1.56 \times 10^5\,M^{-1}\,cm^{-1}$ at 535 nm, as previously described (Girotti *et al*., 1991). Advanced oxidation protein products (AOPP) were detected spectrophotometrically at 340 nm and expressed as chloramine-T equivalents (μmol $\times L^{-1}$) (Selmeci *et al*., 2005). The redox balance was measured according to a previously published method (Alamdari *et al*., 2007) with modifications. This method can measure the balance of oxidants and antioxidants simultaneously, by using 3, 3′, 5, 5′-tetramethylbenzidine (TMB) and two different kinds of reactions; one enzymatic reaction in which the chromogen TMB is oxidized to a color cation by peroxides, and a chemical reaction in which the TMB cation is reduced to a colorless compound by antioxidants, and gives a redox stress index. The photometric absorbance is then compared with the absorbances given by a series of standard solutions that are made by mixing varying proportions (0–100%) of 250 μmol/L hydrogen peroxide and 10 mmol/L uric acid. Hydrogen peroxide and uric acid do not interact with each other, so this mixture has been chosen as representatives of oxidants and antioxidants, respectively. Values are expressed in arbitrary HK (Hamidi-Koliakos) units, which correspond to the percentage of hydrogen peroxide in the standard solution.

Statistical analysis. All data were analyzed by using the PASW Statistics version 18.0 and MedCalc (version 11.4 Software, Belgium) software. Normality of data was assessed with the Kolmogorov–Smirnov test. Since the observed variables did not show significant departures from normal distribution, no data transformation was employed. Physical characteristics and nutrition habits of the tested individuals before supplementation were tested with the unpaired *t*-test. The χ^2-test was used to compare distributions of phenotypes in the two groups. The effects of supplementation and training were analyzed by an analysis of variance (ANOVA) with repeated measures to test for the two main effects and for the interaction between them. When a significant *p*-value was obtained, Bonferroni *post-hoc* comparison test was employed to determine the differences between groups. Two-tailed *p* values are given throughout.

RESULTS

Subject's physical characteristics are listed in the Table 1. There were no significant differences between the two groups with respect to these characteristics at baseline ($p > 0.05$, *t*-test).

The dietary data is presented in Table 2. The supplemented and placebo group did not differ in estimated energetic and nutritional intake ($p > 0.05$, *t*-test). The dietary analysis obtained from the 4-day food diary showed that the mean vitamin A and E intakes were below the dietary reference intake recommendations for the Asx and P groups (Food and Nutrition Board, 2000).

Table 1. Physical characteristics of soccer players

	Astaxanthin (n = 21)	Placebo (n = 19)
Age (year)	17.91 ± 0.16	17.62 ± 0.14
Weight (kg)	70.9 ± 1.7	72.3 ± 1.8
Height (cm)	177.9 ± 1.4	180.2 ± 1.4
Body mass index (kg/m2)	22.37 ± 0.33	22.24 ± 0.41
Fat (%)	9.41 ± 0.74	9.72 ± 0.81
VO_{2max} (ml/min/kg)	55.5 ± 1.2	52.9 ± 0.7
Training experience (years)	10.23 ± 0.32	9.88 ± 0.21

Values are expressed as mean ± SEM.

Table 2. Estimated daily energy and nutrient intake of soccer players

	Astaxanthin (n = 21)	Placebo (n = 19)
Energy (kcal)	3154 ± 247	2932 ± 147
Protein (g)	124 ± 8.7	125 ± 6.3
Carbohydrates (g)	412 ± 33	366 ± 23
Monosaharides (g)	109 ± 12	123 ± 18
Fiber (g)	13.2 ± 1.4	12.6 ± 1.5
Fat (g)	104 ± 9	101 ± 7
Saturated fat (g)	33.2 ± 3.5	29.9 ± 3.2
Cholesterol (mg)	328 ± 23	344 ± 38
Vitamin A (IU)	2312 ± 442	2120 ± 389
Vitamin C (mg)	149 ± 25	135 ± 30
Vitamin E (mg)	5.4 ± 0.7	6.1 ± 1.5
Copper (mg)	2.3 ± 0.4	1.92 ± 0.5
Iron (mg)	14.4 ± 0.9	15.1 ± 1.6
Manganese (mg)	4.9 ± 0.3	4.0 ± 0.9
Selenium (μg)	187 ± 15	164 ± 8
Zinc (mg)	13.0 ± 1.4	13.5 ± 1.8

Values are expressed as mean ± SEM.

Lipid profiles of soccer players are shown in Table 3. All parameters measured remained within their normal laboratory reference ranges at all time points during the study. The ANOVA analysis showed a significant effect of training on CHOL, HDL-C and LDL-C ($p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively). In both the Asx and P group, there was a decrease of HDL-C after 45 days, followed by an increase at the end of the study. On the contrary, CHOL and LDL-C increased after 45 days and decreased by the end of the study in both groups of soccer players. No significant effect of training or supplementation on TG was observed.

PON1 activities toward substrates paraoxon and diazoxon are presented in Fig. 1. The significant interaction effect of supplementation and training ($p < 0.05$) on POase activity was observed, while a significant main effect of training ($p < 0.05$) was recorded regarding the DZOase activity. Also, the DZOase activity increased in Asx group (42%) after 90 days ($p < 0.01$, Bonferroni test), while there was no significant difference in the P group ($p > 0.05$).

There was a difference between the PON1 192 Q and R polymorphism distribution in the supplemented and P group, as it could be seen in Fig. 2 ($\chi^2 = 3.987$; $p = 0.136$).

Table 3. Lipid status of the soccer players at baseline, after 45 days and after 90 days of regular training and supplementation

	Astaxanthin (n = 21)			Placebo (n = 19)			ANOVA		
	Baseline	45 day	90 day	Baseline	45 day	90 day	S	T	SxT
CHOL (mmol/L)	4.28 ± 0.20	4.61 ± 0.22*	4.39 ± 0.24	4.54 ± 0.23	4.67 ± 0.26*	4.50 ± 0.27	ns	<0.05	ns
HDL-C (mmol/L)	1.27 ± 0.05	1.21 ± 0.05*	1.30 ± 0.05	1.29 ± 0.07	1.19 ± 0.06	1.31 ± 0.06#	ns	<0.01	ns
LDL-C (mmol/L)	2.63 ± 0.19	2.98 ± 0.20*	2.73 ± 0.20	2.97 ± 0.24	3.13 ± 0.24	2.91 ± 0.25#	ns	<0.05	ns
TG (mmol/L)	0.84 ± 0.12	0.91 ± 0.08	0.80 ± 0.08	0.95 ± 0.14	0.80 ± 0.09	0.70 ± 0.09	ns	ns	ns

Values are expressed as mean ± SEM. S-supplementation, T-training, SxT-training and supplementation interaction effect. The difference in relation to baseline was significant at $p < 0.05$ (*). The difference in relation to 45 day was significant at $p < 0.05$ (#).

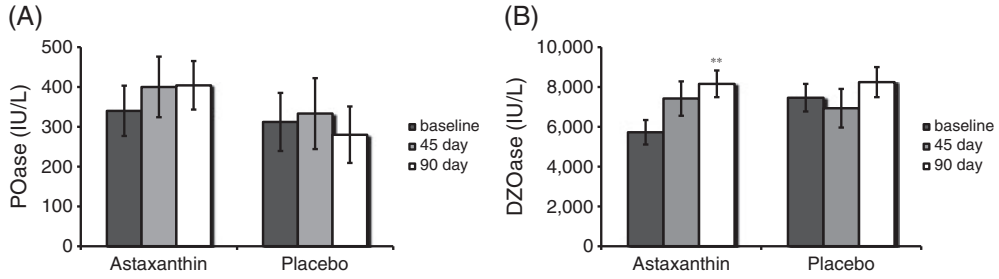

Figure 1. POase activity (A) and DZOase activity (B) at baseline, after 45 days and after 90 days of supplementation in astaxanthin and placebo groups. Values are expressed as mean±SEM. The difference in relation to baseline was significant at $p<0.01$ (**).

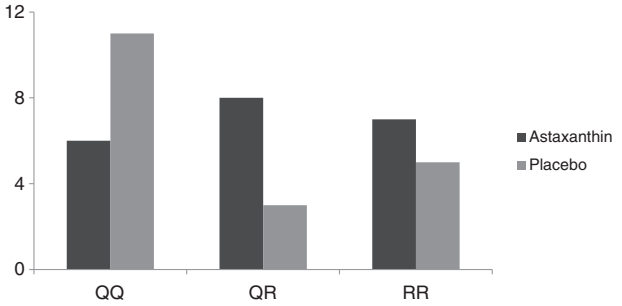

Figure 2. PON1 192 Q and R polymorphism distribution in two groups of soccer players. PON1Q192, PON1QR192 and PON1R192 phenotypes were determined according to the measured DZOase/POase activity ratio.

There was no correlation between HDL-C levels and PON1 activity either in Asx or P group at any time point.

The ANOVA with repeated measures showed a significant interaction effect of supplementation and training ($p < 0.05$), as well as significant main effect of training ($p < 0.01$) on total –SH groups content (Fig. 3A). *Post-hoc* comparison revealed significant increase of total –SH groups content in Asx group after 45 and 90 days, when compared to baseline values ($p < 0.05$, $p < 0.001$, respectively, Bonferroni test).

Regarding the TBARS levels (Fig. 3B), we observed a significant main effect of training ($p < 0.001$). In both Asx and P groups, there was a significant decrease of TBARS after 45 days ($p < 0.001$ and $p < 0.05$,

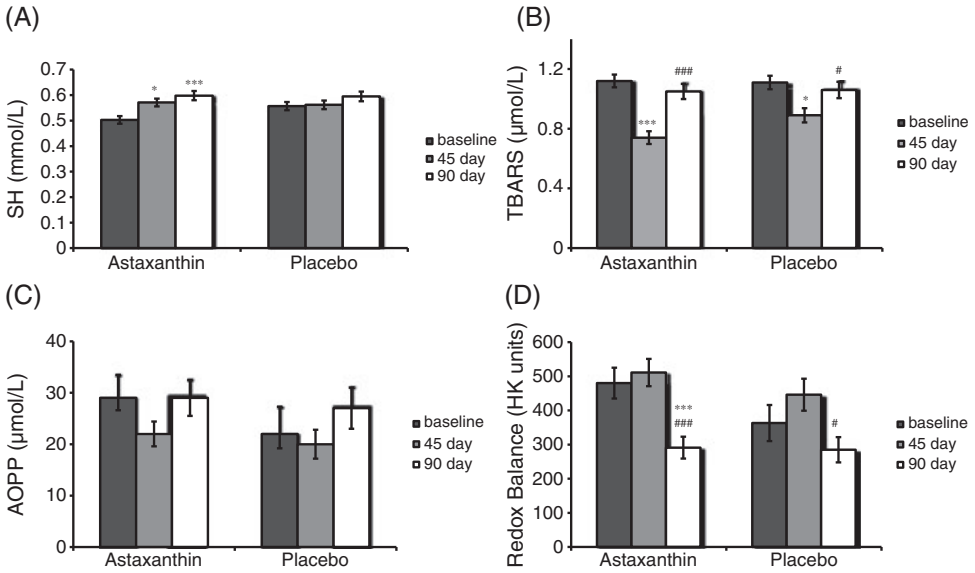

Figure 3. Total SH groups content (A), TBARS levels (B), AOPP levels (C) and redox balance (D) at baseline, after 45 days and after 90 days of supplementation in astaxanthin and placebo groups. Values are expressed as mean±SEM. The difference in relation to baseline was significant at $p<0.05$ (*) and $p<0.001$(***). The difference in relation to 45 day was significant at $p<0.05$ (#) and $p<0.001$ (###).

respectively, Bonferroni test), followed by an increase at the end of the study ($p < 0.001$ and $p < 0.05$, respectively, Bonferroni test). After 45 days of supplementation, we observed significantly lower TBARS levels in supplemented group compared to placebo group ($p < 0.05$), with no differences in TBARS levels after 90 days of supplementation.

Although the changes in AOPP level showed the similar pattern as for the TBARS level during the examined period (Fig. 3C), there was no significant effect of supplementation or training ($p > 0.05$).

The ANOVA analysis showed a significant main effect of training ($p < 0.001$) on redox balance (Fig. 3D). *Post-hoc* analysis revealed significant decrease of redox balance in Asx group after 90 days in comparison to baseline values ($p < 0.001$, Bonferroni test) and 45 day ($p < 0.001$, Bonferroni test). Redox balance in P group also significantly decreased by the end of the study compared to 45 day ($p < 0.05$, Bonferroni test).

At the beginning of the study, we found lower DZOase activity and total SH groups content, and higher redox balance in Asx compared to the P group, with a marginally significant difference ($p = 0.073$, $p = 0.060$ and $p = 0.080$, respectively).

DISCUSSION

The present study investigates, for the first time, the effect of Asx supplementation on PON1 activity and oxidative stress status in young elite soccer players, while following their habitual dietary pattern, training and competition program.

The role of regular training on PON1 activity has not been well established yet. It was reported that regular physical activity might increase PON1 activity (Senti *et al*., 2003; Arslan *et al*., 2005). One of the possible explanations is that regular physical activity causes a repeated increase in ROS, which hypothetically acts as an inducer of transcription of endogenous antioxidant genes, particularly PON1 (Sun and Oberley, 1996). However, some studies have failed to show an increase in PON1 activity in subjects who practiced regular physical activity (Richter *et al*., 2005; Brites *et al*., 2006). In addition, regular training up to 8 weeks did not have significant effects on PON1 activity (Gharakhanlou *et al*., 2007; Martinovic *et al*., 2011). In accordance, our results showed that soccer training over the 90 days of observational period did not affect basal PON1 activity toward paraxon or diazoxon. It is possible that PON1 activity already reached its maximum due to an adaptive response induced by regular soccer training over 10 years, as it has been shown in female volleyball players (Martinovic *et al*., 2009).

Supplementation with Asx during regular soccer training had a beneficial effect in terms of improving POase activity. In addition, the Asx group experienced increased DZOase activity (Fig. 1). This is in agreement with previously published data showing that dietary factors and antioxidant intake can have a modulatory effect on PON1 activity. Jarvik *et al*. (2002) have demonstrated that vitamin C intake is associated with an increase in PON1 activity. Aviram *et al*. (2004) found significant increase in PON1 activity in patients with carotid stenosis after pomegranate juice consumption, rich in polyphenolic antioxidants. Tsakiris *et al*. (2009) showed that alpha-tocopherol supplementation has beneficial effect on serum PON1/arylesterase activities in healthy individuals. It is well known that PON1 has a free cysteine residue (Cys284) and that mutating Cys284 to alanine or serine residues decreased, but not abolished the enzyme paraoxonase and arylesterase activities (Sorenson *et al*., 1995). It was reported that exposition of PON1 to hydroxyl radical and superoxide anion induced a significant decrease in PON1 activity as well as a reduction in the number of PON1 free thiol groups (Jaouad *et al*., 2006). We suggest that increase in PON1 activity toward paraoxon and diazoxon activity as a result of Asx supplementation might be a consequence of protection from oxidative modification of PON1 free thiol group in active center of the enzyme.

In addition, soccer players supplemented with Asx in our study experienced a significant increase in total SH group content, while no significant changes were observed in the P group (Fig. 3A). Considering that exercise induced-oxidative stress is associated with decline in total SH groups content (Bonina *et al*., 2005; Zembron-Lacny *et al*., 2009), these results indicate that Asx might protect SH groups against oxidative modifications. A previous study has also observed the beneficial effect of antioxidant supplementation on the serum level of SH groups (Bonina *et al*., 2005).

The limitation of the current study is unequal phenotype distribution in both the supplemented and placebo group (Fig. 2). At the beginning of the study, DZOase activity and total SH groups content were lower, and redox balance was higher in Asx compared to P group, with marginally significant difference. DZOase activity and SH groups content might be reduced due to an increased redox balance observed in the Asx group. Strong association of redox balance with reduced DZOase activity was previously reported in pregnant women (Stefanovic *et al*., 2012). However, by the end of the study, there was no difference in these parameters between two groups. Asx supplementation might help in reducing redox balance and restoring DZOase activity and SH groups content in subjects more susceptible to oxidative stress.

Previous investigations reported favorable lipid profile of athletes compared to non athletes as evidenced by lower concentrations of TG (Nikolaidis *et al*., 2003; Popovic *et al*., 2010), CHOL (Nikolaidis *et al*., 2003) or LDL-C (Popovic *et al*., 2010) and higher levels of HDL-C (Brites *et al*., 2000; Evelson *et al*., 2002). On the contrary, in the present study, HDL-C decreased and LDL-C increased after 45 days of regular training, returning to baseline values at the end of the study (Table 3). Despite the fact that physical activity has a beneficial effect on lipid profile, it is possible that soccer training might cause mild, temporary disturbance in the lipid profile of young athletes.

Several studies have reported beneficial effect of Asx on lipid status (Yuan *et al*., 2011), but the results of the present study did not show any significant effect. The possible explanation may be that people included in the study are young, healthy and physically active, with lipid profile parameters within the normal reference range.

The association of PON1 with HDL-C is important for maintenance of normal serum enzyme activity. However, PON1 is associated exclusively with a discrete subpopulation of HDL particles, which explains the poor correlation between PON1 and HDL-C found in the present study (James and Deakin, 2004).

The soccer training and competition sessions might increase oxidative stress at rest, indicated by the increased MDA plasma levels, as reported by previous studies (Zoppi *et al*., 2006; Tauler *et al*., 2008). Present investigation also showed significant effect of regular soccer training on TBARS levels in young athletes (Fig. 3B). Observed higher TBARS levels at baseline reflect the high physical stress soccer players are exposed to and the increase in TBARS levels from day 45 to day 90 may be due to the more intensive exercise sessions in the second half of the season, as well as cumulative augment through the season (Fig. 1).

Previous studies showed strong antioxidant activity of Asx against lipid peroxidation and oxidative stress (Tripathi and Jena, 2009; Chan *et al*., 2009; Choi *et al*., 2011). In the present study, we observed significantly lower TBARS levels in supplemented group compared to controls after 45 days of study period, while there was no difference in TBARS levels after 90 days of supplementation. It is possible that Asx supplementation might reduce lipid peroxidation induced by soccer training of lower intensity in the first half of competitive season, but not during the period of more intensive physical activity.

The TBARS assay is well accepted for determination of lipid-derived decomposition products, but certain methodological problems must be discussed. This biomarker estimates the levels of a range of lipoperoxidation aldehydes secondary to lipid peroxidation, but lipoproteins and protein bound aldehydes can also react with TBA. Several other compounds, including sugars, amino acids and bilirubin, also interfere(Meagher and Fitzerald, 2000). One previous study showed that antioxidant supplements significantly influenced lipid hydroperoxide and F2-isoprostane levels in response to exercise, whereas they did not alter MDA measured by TBARS assay (Childs *et al*., 2001).

Changes in AOPP levels showed the similar pattern as for the TBARS levels during the examined period in both groups of soccer players (Fig. 3C), but without statistical significance. Other studies have also reported no changes in protein oxidation during 3 months of regular soccer training (Zoppi *et al*., 2006; Tauler *et al*., 2008). AOPP is considered as a reliable biomarker of oxidant-mediated protein damage following long-term oxidative stress (Neubauer *et al*., 2008). It is possible that the training program applied in the present study was not intensive and/or long enough to cause significant protein oxidation. In addition, young soccer players analyzed in the present study had lower AOPP levels compared to the values observed in other studies (Martinovic *et al*., 2009; Pialoux *et al*., 2009).

Redox balance is a novel parameter, which determines the prooxidant burden and the antioxidant capacity in a single assay. In the present study, we observed significant decrease in redox balance as a result of regular training in both groups of soccer players (Fig. 3D). Our results suggest that soccer training may result in the suppression of free radical production, due to an up regulation in the body's antioxidant defense system. The oxidative stress status reverts back to a normal state as a result of adaptation, despite the fact that products of oxidative modifications are still present.

CONCLUSIONS

Asx supplementation had a beneficial effect in improving PON1 activity toward paraoxon and diazoxon, as well as total SH groups content in young soccer players. Asx might be of special interest for the athletes who are more susceptible to oxidative stress, providing additional support for enzymatic and non-enzymatic endogenous antioxidant defense systems in order to attenuate increases in ROS production. However, further research is required to confirm our assumptions and collate more information regarding the effect of Asx supplementation in athletes.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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