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The Influence of Supplementation With Artichoke (*Cynara scolymus L.*) Extract on Selected Redox Parameters in Rowers

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High-intensity physical exercise decreases intracellular antioxidant potential. An enhanced antioxidant defense system is desirable in people subjected to exhaustive exercise. The aim of this study was to investigate the influence of supplementation with artichoke-leaf extract on parameters describing balance between oxidants and antioxidants in competitive rowers. This double-blinded study was carried out in 22 members of the Polish rowing team who were randomly assigned to a supplemented group (n = 12), receiving 1 gelatin capsule containing 400 mg of artichoke-leaf extract 3 times a day for 5 wk, or a placebo group (n = 10). At the beginning and end of the study participants performed a 2,000-m maximal test on a rowing ergometer. Before each exercise test, 1 min after the test completion, and after a 24-hr restitution period blood samples were taken from antecubital vein. The following redox parameters were assessed in red blood cells: superoxide dismutase activity, glutathione peroxidase activity, glutathione reductase activity, reduced glutathione levels, and thiobarbituric-acid-reactive-substances concentrations. Creatine kinase activity and total antioxidant capacity (TAC) were measured in plasma samples, lactate levels were determined in capillary blood samples, and serum lipid profiles were assessed. During restitution, plasma TAC was significantly higher ($p < .05$) in the supplemented group than in the placebo group. Serum total cholesterol levels at the end of the study were significantly ($p < .05$) lower in the supplemented group than in the placebo group. In conclusion, consuming artichoke-leaf extract, a natural vegetable preparation of high antioxidant potential, resulted in higher plasma TAC than placebo but did not limit oxidative damage to erythrocytes in competitive rowers subjected to strenuous training.

Keywords: antioxidants, TBARS, exhaustive exercise, training

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There is growing body of evidence (Aslan, Sekeroglu, Tarakcioglu, Bayýroglu, & Meral, 1998; Franzoni et al., 2005) to indicate that physical training can increase antioxidant potential in athletes. Enhanced antioxidant potential might protect against damages associated with exercise-induced oxidative stress. It should be emphasized, however, that the human organism is capable of maintaining stable balance between oxidants and antioxidants only if intensity and duration of exercise are moderate. Exercise of higher intensity is associated with uncontrolled generation of reactive oxygen species (ROS), which inflicts damage on DNA and proteins and decreases fluidity and permeability of cell membranes as a result of lipid peroxidation (Goto et al., 2003; Marzatico, Pansarasa, Bertorelli, Somenzini, & Della Valle, 1997). Increasing intensity of exercise leads to gradual exhaustion of antioxidant potential, which manifests in decreased activity of intracellular antioxidant enzymes and diminished levels of extracellular nonenzymatic antioxidants (Aslan et al.; Liu et al., 2000). According to Kretzschmar and Klinger (1990) and Venditti, Masullo, and Meo (1999), these changes are sensitive markers of enhanced peroxidation processes in humans.

Although training results in adaptations that increase the defensive potential against ROS, these changes might be insufficient to prevent oxidative damages after maximal-intensity exercise (Bloomer, Goldfarb, & McKenzie, 2006; Margonis et al., 2007). Similarly, varied nutrition might be insufficient to supply enough antioxidants to prevent oxidative stress during exhaustive exercise.

High-intensity oxidative stress induced by maximal exercise indicates a need for an increased supply of antioxidants in the diet of those exposed to strenuous exercise (Bloomer et al., 2006; Pilaczynska-Szczesniak, Skarpañska-Stejnborn, Deskur, Basta, & Horoszkiewicz-Hassan, 2005). There are several arguments for enriching athletes' diet in vegetable antioxidants; for example:

- Higher assimilation capacity of natural products than synthetic preparations
- Impossibility of overdose
- Interactions between various antioxidants to neutralize ROS

It has been reported that artichoke has antioxidative and antiatherogenic properties. Artichoke inhibits cholesterol synthesis de novo in the liver, prevents LDL modification, stimulates bile secretion, and has strong antioxidant properties (Gebhardt, 1997a; Wolf, 1996). The antioxidant properties of artichoke result from its high content of chlorogenic acid, cynarine, and flavonoids—derivatives of luteoline (cynaroside, scolimoside, and cynarotriozyd; Brown & Rice-Evans, 1998; Gebhardt, 1997b; Llorach, Espin, Tomas-Barberan, & Ferreres, 2002). According to Gebhardt (1997a), the antioxidant potential of artichoke depends not only on properties of particular substances but also on the quantitative and qualitative composition of the extract.

The aim of the current study was to investigate the influence of artichoke-leaf extract on lipid profile and intensity of exercise-induced oxidative stress in rowers participating in a training camp.

Material and Methods

Study Population

The study population consisted of 22 male members of the Polish rowing team for the Youth World Championships 2006 in Hazelwinkel, Belgium. The study was performed in June and July during a 5-week training camp between the preparation and competition periods. The participants' characteristics are presented in Table 1. Data concerning training profile, including intensity, volume (in minutes), and type (specific: rowing endurance, technique, speed, etc.; semispecific: rowing ergometer; nonspecific: jogging, strength), were recorded daily. All training data were analyzed for intensities below and above the lactate threshold of 4 mmol/L, as shown in Figure 1 as extensive (below lactate threshold) and intensive (above lactate threshold) workload.

During the entire study period, athletes were residents in one of the Olympic Games training centers and took meals exclusively in the center. Their regular menu consisted of a mixed diet containing the recommended dietary allowance of carbohydrates, proteins, fats, and micronutrients (vitamins and minerals). Daily food and caloric intake, as well as fruit and vegetable intake, of the participants did not change over the study period. The athletes informed the scientific staff that they had not been taking any drugs, medication, or nutritional supplements for 2 weeks before and during the study.

Experimental Procedure

Athletes enrolled in the study were randomly assigned to receive artichoke-leaf extract ($n = 12$), or placebo ($n = 10$). The rowers in the supplemented group were given an artichoke preparation (Europlant PhytoPharm Kleka SA, Poland) three times a day for 5 weeks. One gelatin capsule contained 400 mg of dry artichoke-leaf extract (*Cynarae folii extractum aq. siccum [4–6: 1]*), which corresponds to 2 g of raw material. The rest of the preparation was composed of the following inactive

Table 1 Basic Characteristics of the Studied Groups, *M* ± *SD*

Parameter	Supplemented group ($n = 12$)	Control group ($n = 10$)
Age (years)	20.8 ± 0.9	19.1 ± 0.2
Body mass (kg)	88.4 ± 5.3	84.3 ± 6.6
Body height (cm)	194.3 ± 5.9	189.3 ± 8.2
Years of training (years)	5.4 ± 1.1	4.7 ± 1.7
Sport class (*n*)		
country master class	6	7
Class I	6	3

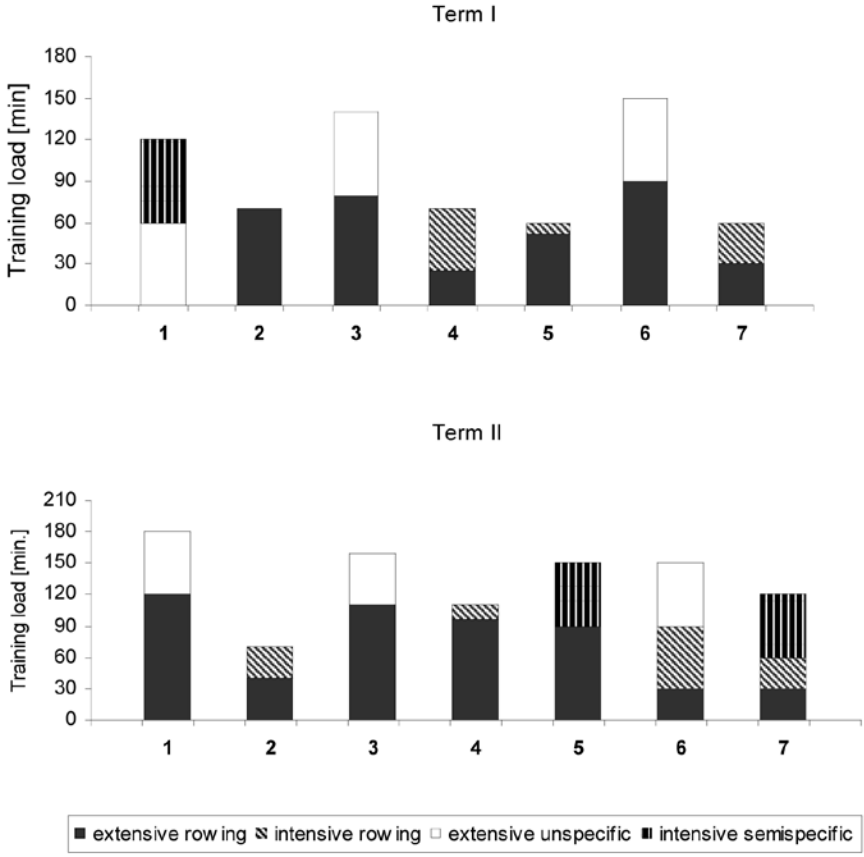

Figure 1 — Training schedule during the week preceding blood-sample collection before (Term I) and after (Term II) the supplementation period (volume in min/day).

ingredients: lactose, talc, magnesium stearate, colloidal silica, and cornstarch. At the same time and with the same dosage regimen, participants in the placebo group received dyed gelatin capsules containing inactive ingredients. All participants were informed of the nature of the investigation and gave their written consent to participate in the study according to the requirements of the local ethical committee.

On the first day (before supplementation) and at the end of the training camp (after supplementation), the athletes performed a controlled 2,000-m rowing exercise test. Each participant had to cover the distance on a rowing ergometer (Concept II, USA) in as short a time as possible. Before the test participants performed 5-min individual warm-ups.

Sample Treatment

Blood samples for redox parameters were taken from the antecubital vein, with K_2EDTA (dipotassium ethylene diamine tetra acetic acid) used as an anticoagulant before each incremental exercise test (in the morning, after an overnight fast), 1 min after test completion, and after a 24-hr recovery period. Samples were centrifuged immediately to separate red blood cells from plasma. Packed erythrocytes were washed three times with saline and lysed with ice-cold, redistilled water. Plasma, serum, and lysed erythrocytes were frozen immediately and stored at –28 °C until use (up to 1 week). In addition, capillary blood samples were taken by finger prick before and after each exercise test to assess lactate levels.

Measurements

Total antioxidant capacity (TAC), used as an overall measure of plasma antioxidant capacity, was assessed with a commercially available kit (Randox-TAS, Cat. No. NX 2332, UK). This assay was based on interaction between chromogen (2,2′-azino-di-[3-ethylbenzthiazoline sulphonate], ABTS°) and ferrylmyoglobin, a free radical formed in the reaction of metmyoglobin and hydrogen peroxide. Antioxidants in added plasma scavenge ABTS° and prevent absorbance to a degree related to the overall plasma antioxidant capacity. This radical had a stable green color, measured at 600 nm. Antioxidants contained in the plasma suppress the color development proportionally to the amount of antioxidants in the sample.

Superoxide dismutase (SOD) activity was measured in washed erythrocytes after their lysis by means of a commercially available kit (Randox-Ransod, Cat No. SD 125, UK). SOD catalyzes dismutation of superoxide anion (O_2), leading to formation of oxygen and hydrogen peroxide. The determination of SOD activity was based on the production of O_2 by the xanthine and xanthine oxidase system. Superoxide anions reacted with the 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The units of SOD activity were calculated on the basis of changes in the absorbance over 3 min, at 505 nm and 37 °C, and from data from the standard curve generated with known amounts of purified SOD, which was obtained from the manufacturer. The SOD activity was expressed in U/g Hb.

Glutathione peroxidase (GPx) activity in the hemolysate samples was measured using a commercially available kit (Randox-Ransel, Cat No. RS 506, UK). GPx catalyzes the oxidation of reduced glutathione in the presence of cumene hydroperoxide. The rate of glutathione oxidation was measured by monitoring the disappearance of $NADPH+H^+$ in the reaction medium, because $NADPH+H^+$ was consumed to reduce oxidized glutathione by glutathione reductase. The decrease in absorbance was measured at 340 nm and 37 °C. GPx activity was expressed in U/g Hb.

Glutathione reductase (GR) activity in the hemolysate samples was assayed using reagent from Randox Laboratories (Cat. No. GR 2368, UK). GR catalyzes reduction of oxidized glutathione in the presence of NADPH, which is oxidized to $NADP^+$. For the GR assay, the 50% hemolysates were centrifuged to remove stroma, and 100 µl were diluted with 1.9 ml of 0.9% NaCl (total dilution of the

hemolysates for the GR assay, 1:40). The decrease in absorbance at 340 nm was measured at 37 °C. GR activity was expressed in U/g Hb.

Erythrocyte glutathione (GSH) content was assayed spectrophotometrically using a commercially available kit (GSH-400, Bioxytech, Oxis International, Portland, OR). Briefly, 200 µl of erythrocyte pellet were pipetted and extracted in 800 µl of 6.25% metaphosphoric acid. The extract was vortexed and centrifuged. Test tubes contained 200 µl of supernatant; we added 700 µl of potassium phosphate buffer (pH 7.8) containing 0.2 mM diethylenetriaminepenta-acetic acid and 0.025% lubrol, 50 µl of 1.2×10^{2} M chromogen (4-chloro-1-methyl-7-trifluoromethyl-1-quinolinium sulfate) in 0.2 N HCl, and 50 µl of 30% NaOH. After vortexing, the solution was incubated for 10 min at 25 °C in darkness. The optical density was estimated at 400 nm. A standard curve was obtained with reduced GSH dissolved in 5% metaphosphoric acid.

Concentrations of thiobarbituric-acid-reactive substances (TBARS) in the hemolysate samples were assessed as a measure of oxidative damage to red blood cells. TBARS concentrations were evaluated with the method described by Buege and Aust (1991), involving the acidic breakdown of lipid peroxides into malondialdehyde molecules. Malondialdehyde subsequently reacted with thiobarbituric acid, producing substances suitable for spectrophotometric detection. The tested samples contained 0.025 ml of hemolysate, 0.5 ml of thiobarbituric acid solution (0.375 g/100 ml in 0.25 mol/L hydrochloric acid), 0.5 ml of trichloroacetic acid solution (15 g/100 ml trichloroacetic acid in 0.25 mol/L hydrochloric acid), and 0.475 ml of water. The blank samples contained 0.025 ml of hemolysate, 0.5 ml of trichloroacetic acid solution, and 0.975 ml of water. All samples were mixed vigorously and heated for 15 min in boiling water. Next, they were cooled in ice-cold water and centrifuged at 2,500 *g* for 15 min. The absorbance of the supernatant was determined at 535 nm and 37 °C. The absorbance of the blank sample was subtracted from the absorbance of the tested sample, and the concentrations of TBARS were determined from a standard curve generated with known amounts of tetramethoxypropane. The concentrations of TBARS (malondialdehyde equivalents) were expressed in µmol/g Hb.

Creatine kinase activity was determined in plasma samples with a commercially available kit (Dr Lange, Cat. No. LCN 282, Germany). The results were expressed in U/L.

Concentration of hemoglobin in hemolysate and whole blood were assessed using the cyanmethemoglobin method with Drabkin's reagent and maximal absorbance at 540 nm. The results were expressed in g/100 ml.

The lactate levels in capillary blood were determined immediately after collection of samples using a commercially available kit (Dr Lange, Cat. No. LKM 140). Lactate concentration was expressed in mmol/L.

Serum concentrations of glucose, total and HDL cholesterol, and triglycerides were determined by enzymatic procedures using a commercially available test kit (Cormay). LDL cholesterol was calculated using Friedewald's formula.

Statistical Analysis

Statistical analyses were performed with the STATISTICA v. 7.0 software package. The data were compared using 2 (supplemented and placebo groups) × 3 (times of measurement) repeated-measures analysis of variance (ANOVA). When significant

changes in ANOVA tests were observed, data were additionally analyzed with paired and unpaired Student's *t* tests, with Scheffé's post hoc test for multiple comparisons. All values were reported as $M \pm SD$. Statistical significance was set at $p < .05$.

Results

Table 1 shows physical characteristics of the study population. Participants in the two groups were similar with respect to mean age, height, body mass, and years of training.

Changes in serum lipid profiles and glucose levels are shown in Table 2. Plasma concentrations of HDL-cholesterol, LDL-cholesterol, triglycerides, and glucose did not change over the studied period. Plasma total cholesterol concentrations, however, at the end of the study were significantly lower in rowers in the supplemented group than in the placebo group ($p < .05$).

Training volumes in minutes per day during the week preceding Term I and during the week preceding Term II, specified for extensive rowing, intensive rowing, extensive nonspecific, and intensive semispecific training, are shown in Figure 1. It should be noted that the volume of training was longer and the proportion of training in the high-load phase (>4 mmol/L lactate) was higher before Term II than before Term I.

Data on training loads and lactate levels are shown in Table 3. The data clearly show that the exhaustive exercise resulted in comparable blood lactate levels in athletes receiving artichoke-leaf extract and in the control group. High postexercise

Table 2 Lipid- and Carbohydrate-Metabolism Parameters Before (Term I) and After (Term II) Supplementation, *M* ± *SD*

	Supplemented Group (*n* = 12)		Control Group (*n* = 10)	
Parameter, mg/dL	Before	After	Before	After
Glucose	75.8 ± 6.2	80.6 ± 11.5	80.2 ± 9.5	79.1 ± 3.7
Cholesterol	173.9 ± 17.3	171.9 ± 15.3	176.9 ±14.3	187.1 ± 9.0*
HDL	58.4 ± 6.3	57.5 ± 5.6	59.0 ± 11.9	53.8 ± 2.0
LDL	97.2 ± 15.0	101.3 ± 16.9	106.9 ± 18.8	118.5 ± 11.0
Triglycerides	74.5 ± 15.1	71.6 ± 8.0	66.0 ± 14.6	64.3 ± 7.2

*$p < .05$ relative to supplemented group.

Table 3 Training-Load Lactate Levels Before (Term I) and After (Term II) Supplementation, *M* ± *SD*

	Supplemented Group (*n* = 12)		Control Group (*n* = 10)	
Parameter	Before	After	Before	After
Power (W)	431.6 ± 30.1	445.0 ± 28.6	425.7 ± 18.2	438.5 ± 21.2
Minimum lactate (mmol/L)	1.7 ± 0.4	1.1 ± 0.2†	1.5 ± 0.2	1.2 ± 0.1†
Maximum lactate (mmol/L)	13.6 ± 2.4	16.2 ± 2.3†	14.5 ± 1.5	16.1 ± 1.9
Time (s)	371.0 ± 6.9	367.8 ± 6.7	378.0 ± 7.5	369.3 ± 7.8

†$p < .05$ relative to before supplementation.

lactate concentrations indicate the important contribution of anaerobic metabolism in covering the energetic expense of exercise. Resting lactate levels in both groups were significantly lower at the end of the preparatory camp. In the supplemented group, postexercise lactate concentrations were significantly higher at Term II than at Term I. Other parameters did not differ between Terms I and II.

Table 4 presents comparative analysis of antioxidant potential parameters. The analysis of variance indicates that physical exercise significantly influenced the values of these parameters directly after ergometry and during recovery. Ergometry performed at the beginning of the study yielded similar changes in the antioxidant potential parameters in both groups. After 5-week supplementation with artichoke-leaf extract, plasma total antioxidant capacities before and after exercise and after 24-hr recovery were significantly higher ($p < .01$) in the supplemented group than in the placebo group.

No significant differences in the antioxidant enzymes were observed between the supplemented and the placebo group. SOD activity after recovery showed a slight, nonsignificant tendency toward a decrease (7%) in the supplemented group and toward an increase (18%) in placebo group.

In addition, no significant interaction effect between exercise and artichoke on TBARS and muscle-damage markers was observed (Table 5). After supplementation, the preexercise levels of TBARS in both groups were higher than before supplementation and increased (not significantly) immediately after exercise (by 12% in the supplemented group and 35% in the placebo group). During recovery, TBARS concentrations returned to preexercise values in the supplemented group and remained unchanged in the control group.

Discussion

Incremental maximal exercise tests performed by rowers at the beginning and end of the preparatory camp caused an imbalance between blood oxidants and antioxidants; the severity of this imbalance was similar in the supplemented and placebo groups (Table 5). Increased creatine kinase (CK) activity after the ergometry suggests that exercise-induced oxidative stress disrupted the integrality of cellular membranes. This finding supports the view that lipid peroxidation leads to an upset of the cellular membranes' integrity (Aslan et al., 1998; Kelle, Hüda, Sermet, Atmaca, & Tümer, 1999; Liu et al., 2000; Yuan et al., 2003). Significantly higher resting TBARS levels and CK activity at the end of the camp (Table 5) indicate an important role of exercise intensity. The higher proportion of training in the higher load phase at the end of the preparatory camp (during the week preceding Term II; Figure 1) resulted in more severe homeostasis impairment, as indicated by more than doubled resting TBARS levels and nearly doubled resting CK activity at the end of the preparatory camp. Increased resting TBARS levels and CK activity at Term II most probably resulted from incomplete recovery after an additional 4 hr of exercise. In accordance with our findings, Yuan et al. proved that increased CK activity in plasma is determined by exercise intensity during the last training session and on preceding days. The results obtained by Ilhan, Kamanli, Ozmerdivenli, and Ilhan (2004) suggest that exercise of mixed aerobic–anaerobic character, in contrast to purely aerobic or anaerobic exercise, results in a significant increase in plasma TBARS levels directly after the exercise, as well as 24 and 48 hr thereafter.

Table 4 Parameters of Antioxidant Potential in Supplemented (Su) and Placebo (Pla) Group Before, After, and Recovery

		Before			After			ANOVA		
Parameter		Preexercise *M* ± *SD*	Postexercise *M* ± *SD*	Recovery *M* ± *SD*	Preexercise *M* ± *SD*	Postexercise *M* ± *SD*	Recovery *M* ± *SD*	Ex	Art	Ex × Art
SOD (U/g Hb)	Su	1,537.3 ± 141.8	1,997.9 ± 569.2†	2,153.7 ± 433.9†	1,671.3 ± 188.7	1,952.4 ± 237.8†	1,824.2 ± 122.3†	$p < .001$	NS	NS
	Pla	1,607.2 ± 219.1	1,896.1 ± 262.9†	2,264.7 ± 466.8†	1,670.2 ± 133.1	1,873.1 ± 194.0†	2,216.6 ± 172.4†			
GPx (U/g Hb)	Su	63.4 ± 7.3	74.2 ± 9.6†	74.5 ± 9.2†	61.3 ± 7.3	54.2 ± 5.9†	60.2 ± 7.5	$p < .001$	NS	NS
	Pla	62.0 ± 4.4	71.2 ± 12.3	72.0 ± 5.4†	63.4 ± 5.4	57.7 ± 3.3†	60.7 ± 11.4			
GR (U/g Hb)	Su	4.5 ± 1.0	4.8 ± 1.9	6.8 ± 1.4†	5.1 ± 1.0	5.4 ± 1.2	6.12 ± 1.3	$p < .01$	NS	NS
	Pla	4.3 ± 0.9	4.6 ± 0.9	6.2 ± 1.6†	4.8 ± 1.6	5.2 ± 1.1	6.34 ± 1.1†			
GSH (mmol/L)	Su	3.4 ± 0.3	2.9 ± 0.9	2.8 ± 0.5†	3.8 ± 0.6	3.5 ± 0.6	3.3 ± 0.4	$p < .01$	NS	NS
	Pla	3.7 ± 0.9	2.9 ± 0.9	2.6 ± 0.7†	3.6 ± 0.6	3.4 ± 1.0	3.2 ± 0.6			
TAC (mmol/L)	Su	2.0 ± 0.1	1.6 ± 0.3†	1.4 ± 0.2†	1.7 ± 0.1*	1.6 ± 0.2*	1.6 ± 0.2*	$p < .001$	$p < .01$	$p < .001$
	Pla	1.9 ± 0.1	1.5 ± 0.1†	1.4 ± 0.2†	1.5 ± 0.2	1.5 ± 0.2	1.4 ± 0.2			

Note. Ex = exercise; Art = artichoke; SOD = superoxide dismutase; GPx = glutathione peroxidase; GR = glutathione reductase; GSH = glutathione; TAC = total antioxidant capacity.

*$p < .05$ relative to Pla group. †$p < .05$ relative to preexercise.

Table 5 Markers of Free-Radical Production and Muscle Damage in Supplemented (Su) and Placebo (Pla) Group Before, After, and Recovery

		Before			After			ANOVA		
Parameter		Preexercise *M* ± *SD*	Postexercise *M* ± *SD*	Recovery *M* ± *SD*	Preexercise *M* ± *SD*	Postexercise *M* ± *SD*	Recovery *M* ± *SD*	Ex	Art	Ex × Art
CK (U/L)	Su	92.2 ± 50.4	166.6 ± 92.4†	140.0 ± 47.4†	199.0 ± 134.2	297.3 ± 142.8	231.4 ± 78.3	$p < .05$	NS	NS
	Pla	104.4 ± 70.1	136.4 ± 78.2	129.0 ± 78.4	188.4 ± 63.6	285. 80 ± 152.1	210.6 ± 65.6			
TBARS (μmol/g Hb)	Su	1.0 ± 0.6	2.1 ± 0.4†	2.4 ± 0.5†	1.7 ± 0.7	1.9 ± 0.5	1.7 ± 0.5	$p < .001$	NS	NS
	Pla	1.1 ± 0.4	2.0 ± 0.6†	2.2 ± 0.6†	1.7 ± 0.9	2.3 ± 0.5	2.3 ± 0.7			

Note. Ex = exercise; Art = artichoke; CK = creatine kinase; TBARS = thiobarbituric-acid-reactive substances.

*$p < .05$ relative to Pla group. †$p < .05$ relative to preexercise.

In contrast to resting TBARS and CK levels, which were elevated in both groups at the end of the study, total cholesterol concentrations significantly increased in the placebo group and remained unchanged in the supplemented group (Table 2). The increase in serum cholesterol levels in the placebo group might have been an adaptive reaction to oxidative stress induced by intensive exercise. Bereza, Brewer, and Hill (1985) showed that increased plasma cholesterol might protect red blood cells against oxidative damage. Erythrocyte membrane is a dynamic structure that stays in balance with plasma lipids. When plasma cholesterol levels increase, cholesterol uptake by erythrocytes and incorporation into cellular membrane augments, and cellular-membrane susceptibility to ROS decreases. Aguilo et al. (2003) observed an increase in total cholesterol levels after maximal and submaximal exercise in amateur cyclists, but not in professional cyclists performing long-lasting exercise of moderate intensity. In contrast, serum concentrations of nonenzymatic antioxidants (ascorbic acid and tocopherol) after exercise were significantly higher in professional cyclists, whereas there were no changes in concentration of these vitamins in the group of amateur cyclists. Presumably, the role of cholesterol as a factor protecting erythrocytes against oxidative damage increases if plasma levels of antioxidants are low. The results of Mastaloudis, Leonard, and Traber (2001) and Kelle et al. (1999) support this hypothesis. The lack of increase in total cholesterol levels in the supplemented group at the end of the study might be explained by higher plasma antioxidant capacity in this group or the previously described lipid-lowering effect of artichoke (Hellsten, Tullson, Richter, & Bangsbo, 1997; Petrowicz, Gebhardt, Donner, Schwandt, & Kraft, 1997). Artichoke enhances cholesterol elimination via stimulation of bile secretion and inhibits cholesterol synthesis de novo in hepatocytes (Wolf, 1996). Gebhardt (1997a) suggests that the main mechanism of artichoke's action consists of inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity. HMG-CoA reductase is involved in HMG-CoA metabolism to mevalonate acid, which is the main substrate in endogenous cholesterol synthesis. Decreased cholesterol synthesis in the liver results in diminished serum levels of cholesterol and its metabolites. No significant changes in concentrations of various classes of lipoproteins, however, were observed in our study (Table 2).

After the supplementation period, plasma total TAC at rest, directly after the exercise test, and after 24 hr of recovery was significantly higher in participants receiving artichoke extract than in the placebo group. This effect might be attributable to the antioxidative properties of artichoke. Several in vitro studies (Llorach et al., 2002; Peschel et al., 2006), based on the assessment of 1,1-diphenyl-2-picryl-hydrazyl stable-radical-scavenging ability and on the measurement of metal-ion-reducing ability (FRAP), clearly demonstrated a high reductive potential of artichoke. Artichoke extract was also found to prevent hydroperoxide-induced lipid damage in hepatocytes. The reduction of the amount of malondialdehyde formed after exposure to tert-butyl hydroperoxide depended on the concentration of artichoke extract, and the effect was seen when the concentration of the extract was as low as 1 μg/ml (Gebhardt, 1997a). In our study, TBARS concentrations after exercise and during recovery at Term II were lower (the difference did not reach statistical significance) in the supplemented group (Table 5), thus probably indicating diminished exercise-induced lipid damage in participants receiving artichoke extract. The protective properties of artichoke against ROS perhaps depend

on the content of polyphenolics rich in hydroxyl groups, which are able to chelate iron and copper ions and reduce free-radical generation (Brown & Rice-Evans, 1998; Melidou, Riganakos, & Galaris, 2005). Similarly, it has been observed that chelating effects of flavonoids might protect LDL against oxidative damage (Catapano, 1997). Other authors (Melidou et al.; Vuorimaa, Ahotupa, Irjala, & Vasankari, 2005) proved that the level of oxidation depends on the concentration of malondialdehyde in plasma.

The extent of exercise-induced oxidative damage is determined by the intensity of free-radical generation and the capacity of the antioxidant system. Before supplementation, we observed a significant increase in SOD and GPx activity after the exercise test in both study groups; elevated SOD and GPx activity were still found after the 24-hr recovery (Table 4). In both groups GR activity was significantly higher during restitution than preexercise levels. Postexercise changes in antioxidant enzymes depend on exercise intensity, as well as on the individual's adaptation to exercise. An increase in antioxidant enzymes after ergometry indicates activation of mechanisms protecting against enhanced ROS production in our study population. These mechanisms appeared to be insufficient to prevent exercise-induced oxidative stress in rowers performing ergometry (Table 4), however, as indicated by decreased levels of TAC and diminished concentrations of GSH in erythrocytes. According to Gohil, Vinie, Stanley, Broks, and Packer (1988), submaximal exercise does not change levels of GSH and oxidized glutathione in red blood cells; in contrast, prolonged submaximal exercise decreased GSH concentrations by 60% and increased oxidized glutathione levels by 100% in comparison with preexercise values. Margonis et al. (2007) investigated oxidative-stress markers after a 3-week period of training at various intensities in young men. They found that volume and intensity of training greatly influenced postexercise changes in the balance between oxidants and antioxidants. Training with highest load was associated with a significant decrease in TAC of plasma and reduction of GSH content in whole blood, and markers of free-radical damage increased significantly: carbonyls (73%), TBARS (56%), and oxidized glutathione (25%). It has been suggested that uric acid, which is one of the most potent antioxidants in plasma (Waring et al., 2003), might be taken up by skeletal muscle under an exercise-induced oxidative-stress condition and used as an antioxidant (Hellsten et al., 1997). Therefore, decreased levels of endogenous antioxidants, such as TAC and GSH, might be explained by enhanced involvement of endogenous antioxidants in the defense of cells against oxidative stress.

In the current study, activities of antioxidant enzymes did not differ significantly between the two study groups (Table 4). In both groups SOD activity increased significantly after exercise, both at Term I and at Term II, indicating enhanced generation of superoxide anion. The main (although not only) source of superoxide during exercise of such intensity is reperfusion following ischemia, when a significant amount of well-oxygenated blood perfuses previously ischemic tissues (Frederiks & Bosch, 1995). In addition, elevated body temperature decreases activity of cytochrome oxidase in respiratory chain, thus leading to electron leakage to oxygen to form O_2^-. As a result of oxidative stress and increased calcemia (calcium ion levels; Ca^{+2}), xanthine dehydrogenase is irreversibly converted to xanthine oxidase (Ji, 1999), which uses molecular oxygen (O_2), and not NAD^+, as the final acceptor of electrons.

Jiménez-Escrig, Dragsted, Daneshvar, Pulido, and Saura-Calixto (2003) previously described increased GPx activity and decreased markers of protein damage in plasma and erythrocytes in rats on a diet enriched with artichoke extract. The decrease in GPx activity after the exercise test observed in both of our groups at the end of the preparatory camp (after supplementation) might be explained by higher levels of hydrogen peroxide (H_2O_2); affinity of catalase to hydrogen peroxide is higher than that of GPx. These data indicate that exercise-induced change in GPx activity in erythrocytes depends not only on exercise intensity but also on intensity of training in the period preceding the exercise.

The observed changes in endogenous antioxidant defense potential and levels of free-radical-damage markers reflect the important diversity of training intensity and duration resulting from the assumed plan of training. Supplementation with a natural vegetable preparation containing artichoke-leaf extract resulted in higher plasma TAC than in the placebo group, but it did not influence the level of free-radical-damage markers. Therefore, administration of antioxidants in doses much higher than those used in the current study should be considered during training periods requiring exercise of extremely high intensity.

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