

## Effects of Oral Supplementation With Plant Superoxide Dismutase Extract on Selected Redox Parameters and an Inflammatory Marker in a 2,000-m Rowing-Ergometer Test

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The aim of this study was to investigate the effect of plant superoxide dismutase extract (GliSODin) supplementation on the balance of oxidants and antioxidants in the serum and erythrocytes of competitive rowers. The double-blinded study included 19 members of the Polish rowing team who were participating in a preparatory camp. Subjects were randomly assigned to the supplemented group ( $n = 10$ ), who received 2 capsules (500 mg) of GliSODin extract once daily for 6 weeks, or the placebo group ( $n = 9$ ). At the beginning and end of the study, subjects performed a 2,000-m maximum-effort test on a rowing ergometer. Blood samples were taken from the antecubital vein before each exercise test, 1 min after completing the test, and after a 24-hr restitution period. The following redox parameters were assessed in erythrocytes: superoxide dismutase (SOD) activity, glutathione peroxidase activity, and concentrations of thiobarbituric-acid-reactive substances. In addition, creatine kinase activity and total antioxidant capacity were measured in plasma samples, lactate levels were determined in capillary blood samples, and C-reactive protein and lactate dehydrogenase concentrations were measured in serum. After supplementation, SOD activity was significantly higher ( $p = .0037$ ) in the supplemented group than the placebo group, and C-reactive protein was significantly ( $p = .00001$ ) lower in athletes receiving GliSODin than those in the placebo group. In conclusion, supplementation with an extract rich in SOD activity promoted antioxidant status and protected against increased inflammation in the serum of professional rowers but had no effect on oxidative damage induced by exhaustive exercise.

**Keywords:** antioxidants, exhaustive exercise, athlete

It is well documented that physical exercise is associated with increased formation of reactive oxygen species (ROS; Finaud, Lac, & Filaire, 2006). The main source of ROS is enhanced superoxide production in the mitochondrial electron-transport chain in skeletal-muscle cells. In comparison with resting conditions, oxygen uptake during exercise increases 10–20 times across the whole body (Åstrand & Rodahl, 1986) and 100–200 times in working skeletal muscles (Keul, Doll, & Koppler, 1972). During anaerobic exercise, several additional sources of ROS are involved. These sources include xanthine oxidase production, catecholamine auto-oxidation, and ischemia followed by reperfusion (Kayatekin, Gönenc, Acikgöz, Uysal, & Dayi, 2002). Muscle damage during exercise may further increase ROS formation by activating phagocytic cells recruited to the site of damage (Nikolaidis et al., 2008). This inflammatory response is aimed at destroying injured tissue and repairing it, but it may result in extensive oxygen damage (Pizza, Peterson, Baas, & Koh, 2005).

It has been demonstrated in many studies that exercise may increase antioxidant enzyme activity and modify nonenzymatic antioxidant concentrations (Dékány et al., 2006; Inal, Akyuz, Turgut, & Getsfrid, 2001). However, ROS production during intense exercise overreaches antioxidant capacity (Finaud, Scislawski, et al., 2006; Goto et al., 2003), a phenomenon known as oxidative stress (Sies, 1985). Oxidative stress leads to damage of molecules such as DNA, lipids, proteins, and carbohydrates and may result in cell dysfunction or death (Halliwell & Whiteman, 2004). Therefore, enhancing the endogenous antioxidant defense system may result in reduction of oxidative stress and potentially reduce or prevent cell damage.

The main source of ROS generated during exercise is skeletal muscle. However, because of the invasive nature of muscle biopsy most studies on exercise-induced oxidative stress in humans have looked at changes in redox parameters in the blood (Nikolaidis et al., 2008). Blood carries the substances regarded as oxidative-stress markers (such as thiobarbituric-acid-reactive substances [TBARS]), redistributes antioxidants, and may scavenge long-living ROS (Ghiselli, Serafini, Natella, & Scaccini, 2000). It is thought that changes in the redox parameters in the blood reflect changes in skeletal muscle (Smith, 1995). It has been directly shown that free radicals flow

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out from an active muscle bed in an intensity-dependent manner (Bailey et al., 2004). There are also some data to indicate that erythrocytes and plasma molecules are influenced by exercise-induced oxidative stress (Petibois & Délérís, 2008).

Superoxide dismutase (SOD) is a key component of the intracellular antioxidant defense system. This enzyme catalyzes the reduction of superoxide anions to less reactive hydrogen peroxide. SOD is found in several plants (Perl-Treves & Galun, 1991; Sandalio, López-Huertas, Bueno, & Del Río, 1997), but its use as a diet supplement was limited because of the inactivation of the enzyme in the gastrointestinal tract. Invention of GliSODin has resolved this problem. GliSODin is an original vegetable formula made from a SOD-rich melon extract (*Cucumis melo* LC) that is coupled with the mucosal delivery system formed by the biodegradable gliadin biopolymer and thus can be delivered efficiently by the oral route (Menvielle-Bourg, 2005). The efficacy of GliSODin to increase SOD activity has been demonstrated in clinical trials (Vouldoukis et al., 2003).

In addition, this SOD–gliadin complex has been shown to

- Inhibit oxidative stress (Albicini et al., 2005; Nakajima et al., 2009; Vouldoukis et al., 2004)
- Inhibit ultraviolet oxidative stress (Mac-Mary, Saint-hillier, Courderotmasuyer, Creidi, & Humbert, 2007)
- Promote immune modulation (Okada et al., 2006)
- Inhibit vascular inflammation (Dugas et al., 2003)

Results of a few studies (Arent, DiFabio, Greenwood, Pellegrino, & Williams, 2005; Hong, Hong, Chang, & Cho, 2004) suggested that the melon extract (*Cucumis melo* LC) may attenuate peroxidative and inflammatory response to exercise in athletes.

We hypothesized that supplementation with orally effective vegetable SOD would enhance endogenous antioxidant defense and limit oxidative stress, muscle injury, and inflammatory response during exercise in professional rowers. To this end, we measured levels of selected antioxidants and markers of oxidative damage and inflammation in the blood of rowers performing a 2,000-m time trial before and after 6 weeks of supplementation with GliSODin.

## Materials and Methods

### Study Population

The study population consisted of 19 male members of the Polish rowing team (17 heavyweight and 2 lightweight rowers). Basic characteristics and sport classes of the athletes are shown in Table 1. The study was performed in March to May during a 6-week training camp between the preparation and competition periods. Data on training profile, such as intensity, volume (in minutes), and type (specific—rowing: endurance, technical, speed, etc.; nonspecific—jogging, strength), were recorded

**Table 1 Basic Characteristics of the Studied Groups ( $M \pm SD$ )**

Parameter	Group	
	Supplement ( $n = 10$ )	Control ( $n = 9$ )
Age (years)	21.3 $\pm$ 1.1	20.3 $\pm$ 1.0
Body mass (kg)	89.3 $\pm$ 5.3	86.1 $\pm$ 10.2
Height (cm)	197.2 $\pm$ 3.1	191.8 $\pm$ 6.4
Years of training	7.2 $\pm$ 1.6	5.7 $\pm$ 1.7
Sport class ( $n$ )		
World Master Class	5	1
Country Master Class	5	5
Class I	0	3

Note.  $p > .05$  for all between-group comparisons.

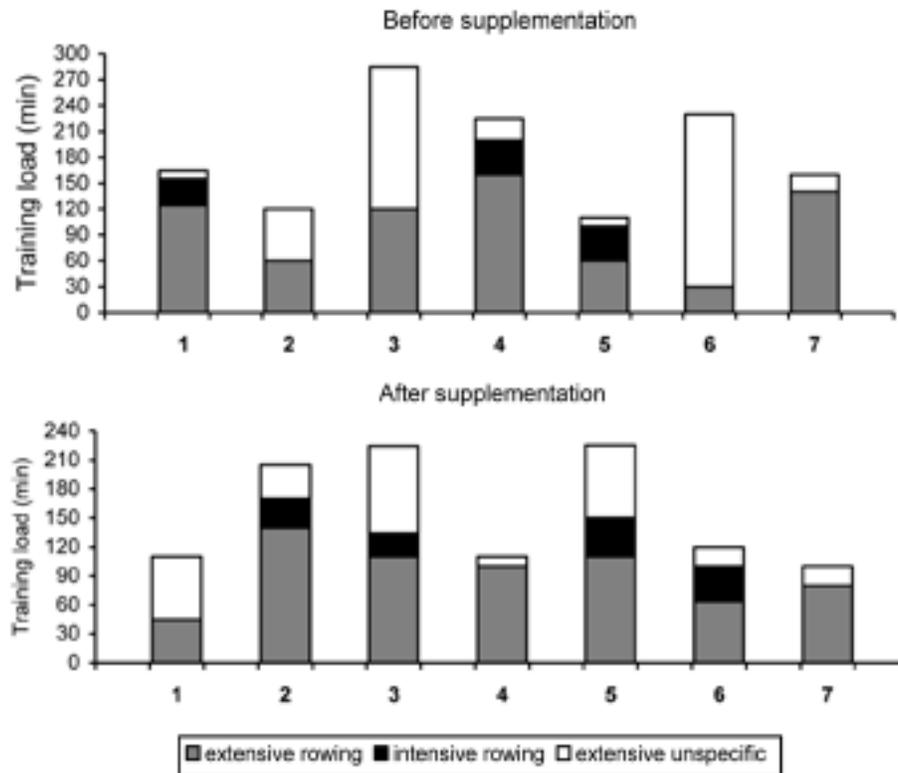
daily. All training data were analyzed for intensities below and above the lactate threshold of 4 mmol/L, as shown in Figure 1, and classified as extensive (below the lactate threshold) or intensive (above the lactate threshold) workload.

Over the entire study period, athletes resided in one of the Olympic Games training centers and took meals exclusively in the center; they were not allowed to consume any food outside the center. Their regular menu consisted of a mixed diet containing the recommended dietary allowance of carbohydrates, proteins, fats, and micronutrients (vitamins and minerals) in conformity with the recommended daily allowance of the Polish Nutrition Societies (Ziemiński, 2001). Daily food and caloric intake, as well as fruit and vegetable intake, of the athletes were constant 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 GliSODin (supplemented group,  $n = 10$ ) or placebo (placebo group,  $n = 9$ ). GliSODin is an original vegetable formula made from a SOD-rich melon extract (*Cucumis melo* LC) coupled with a wheat-based polymer (gliadin). The rowers in the supplemented group took two capsules (500 mg) of GliSODin (produced by Isocell Nutra, France, and supplied by EuroPharma Alliance Sp. z o.o., Wrocław, Poland) once daily for 6 weeks. At the same time, and with the same dosing regimen, subjects in the placebo group took dyed gelatin capsules containing a substance with no significant caloric value or biological activity (maltodextrin).

All subjects were informed of the nature of the investigation and gave their written informed consent to participate in it. The ethics committee at the University



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

School of Medical Sciences in Poznan approved the study protocol.

On the first day (before supplementation) and at the end of the training camp (after supplementation), the athletes performed a controlled 2,000-m time trial, covering the distance on a rowing ergometer (Concept II, USA) in as short a time as possible. Because the results of both tests were taken into consideration during selection of athletes for the championship team, the athletes were well motivated to perform both tests with all-out effort. The subjects were not blinded to any performance measures. Before each test, they performed a 5-min individual warm-up.

### Sample Treatment

Blood samples were taken from the antecubital vein, using dipotassium ethylene diamine tetra-acetic acid as anticoagulant, before each 2,000-m test (in the morning, after an overnight fast), 1 min after completing the test, 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  $-28^{\circ}\text{C}$  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-TAC, Cat. No. NX 2332, UK). This assay was based on the interaction between a chromogen (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate], ABTS $\cdot$ ) and ferrylmyoglobin, a free radical formed in the reaction between metmyoglobin and hydrogen peroxide.

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, leading to the formation of oxygen and hydrogen peroxide. The determination of SOD activity was based on the production of  $\text{O}_2$  by the xanthine and xanthine oxidase system and 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<sup>+</sup> in the reaction medium. GPx activity was expressed in U/g Hb.

Concentrations of 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. The concentrations of TBARS (malondialdehyde equivalents) were expressed in  $\mu\text{mol/g Hb}$ .

Serum levels of C-reactive protein (CRP) were measured with a latex-enhanced immunoturbidometric assay using a System Reagent CRP (latex, Olympus, Melville, NY). CRP reacts specifically with antihuman CRP antibodies on latex particles to yield insoluble aggregates. The absorbance of these aggregates is proportional to the CRP concentration in the sample. The results were expressed in mg/L. The intra-assay coefficient of variation for CRP measurements was 3.5%, and the interassay coefficient of variation was 3.1%.

Lactate dehydrogenase (LDH) activity was measured in plasma. The reagent kits were obtained from Biosystem SA (Cat. No. 11581, Barcelona, Spain). The results were expressed in U/L.

Creatine kinase (CK) 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 was assessed using the cyanmethemoglobin method with Drabkin's reagent and maximal absorbance at 540 nm (Van Kampen & Zijlstra, 1961). The results were expressed in g/100 ml.

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

## Statistical Analysis

Statistical analyses were performed with Statistica v. 8.0 software. Redox parameters, CRP levels, and activity of

CK and LDH were compared using 2 (supplemented and placebo groups)  $\times$  3 (times of measurement) repeated-measures analysis of variance (ANOVA). The distribution of data was analyzed with a Shapiro–Wilk test. If significant changes were observed in ANOVA tests, Fisher's post hoc test was applied to locate the source of significant differences. An unpaired Student's *t* test was used to compare the anthropometric characteristics of the study groups. The results of the 2,000-m tests performed before and after supplementation were analyzed with paired *t* tests for comparisons within the groups and with unpaired *t* tests for comparisons between the groups. All values were reported as  $M \pm SD$ . Pearson's correlation method was used to test the relationship among means. Statistical significance was set at  $p < .05$ .

## Results

Subjects in the supplemented group were similar to the placebo group with respect to mean age, height, body mass, and years of training (Table 1). Mean power output and total time during the 2,000-m test performed at the beginning of the training camp did not differ between the study groups; in both groups blood lactate levels after the exercise test were higher after the training camp than at the first assessment (Table 2).

Training volumes (expressed in min/day) during the week preceding the first term of assessment (before supplementation) and during the week preceding the second term of assessment (after supplementation) specified for extensive rowing, intensive rowing, and extensive nonspecific training are shown in Figure 1. In the load-training phase (before the first assessment), the training volume amounted to 1,295 min/week, of which about 54% was extensive rowing, 38% was nonspecific training such as power training, and 8% was intensive rowing. Total training volume before the second assessment was 1,094 min/week and consisted of approximately 59% extensive rowing, 12% intensive rowing, and 29% land training.

Table 3 shows the comparative analysis of endogenous antioxidant potential parameters. Ergometry performed at the beginning of the study had no influence

**Table 2 Mean Power Output, Blood Lactate Levels, and Total Rowing Time Before and After Supplementation ( $M \pm SD$ )**

Parameter	Supplemented Group ( $n = 10$ )		Control Group ( $n = 9$ )	
	Before	After	Before	After
Power (W)	456 $\pm$ 26	463 $\pm$ 22	431 $\pm$ 31	442 $\pm$ 26
(W/kg)	5.08 $\pm$ 0.14	5.15 $\pm$ 0.10	4.99 $\pm$ 0.43	5.12 $\pm$ 0.47
LA <sub>min</sub> (mmol/L)	1.9 $\pm$ 0.3	1.6 $\pm$ 0.1	1.8 $\pm$ 0.2	2.0 $\pm$ 0.3
LA <sub>max</sub> (mmol/L)	13.5 $\pm$ 1.1	18.2 $\pm$ 4.8*	13.8 $\pm$ 1.3	17.1 $\pm$ 2.5*
Time (s)	366.2 $\pm$ 7.0	364.6 $\pm$ 5.4	371.6 $\pm$ 6.7	370.2 $\pm$ 7.1

Note. LA = lactate levels.

\* $p < .05$  relative to before supplementation.

**Table 3 Changes in Antioxidant Parameters During Exercise Tests Before and After Supplementation (*M* ± *SD*)**

Parameter	Before Supplementation				After Supplementation				Exercise × GIIISODin <i>p</i> = .931
	Preexercise	Postexercise	Recovery	Preexercise	Postexercise	Recovery	Exercise	GIIISODin	
SOD (U/g Hb)							<i>p</i> = .193	<i>p</i> = .0037	
Sup	1,769 ± 223	1,860 ± 173	1,828 ± 316	1,974 ± 186*	2,175 ± 286*	2,104 ± 197*			
Pla	1,768 ± 181	1,880 ± 224	1,833 ± 173	1,780 ± 255	1,983 ± 146	1,807 ± 362			
GPx (U/g Hb)							<i>p</i> = .021	<i>p</i> = .719	<i>p</i> = .960
Sup	52.78 ± 8.59	58.16 ± 9.03	55.51 ± 16.38	54.55 ± 11.95	73.05 ± 17.03†	62.50 ± 12.02†			
Pla	49.04 ± 8.22	57.54 ± 5.78	51.37 ± 11.22	55.60 ± 12.99	69.30 ± 6.51†	59.13 ± 7.24			
TAC (mmol/L)							<i>p</i> = .031	<i>p</i> = .257	<i>p</i> = .292
Sup	1.23 ± 0.24	1.36 ± 0.26	1.24 ± 0.18	1.43 ± 0.90	1.53 ± 0.11	1.48 ± 0.14			
Pla	1.16 ± 0.23	1.18 ± 0.25	1.17 ± 0.28	1.29 ± 0.09	1.50 ± 0.19†	1.42 ± 0.05†			

Note. SOD = superoxide dismutase; Sup = supplemented group; Pla = placebo group; GPx = glutathione peroxidase; TAC = total antioxidant capacity.

\**p* < .05 relative to Pla group. †*P* < .05 relative to preexercise.

on the antioxidant potential parameters in either group. After supplementation, a significant effect of exercise on GPx activity in both groups and on TAC levels in the placebo group was observed. In comparison with preexercise values, GPx activity directly after the exercise test was significantly higher in both groups and after 24-hr restitution in the supplemented group only. TAC levels in the placebo group were significantly increased directly after exercise and after 24-hr restitution compared with the preexercise values.

After 6 weeks of supplementation with an extract rich in SOD, significant effects of GliSODin ( $p = .0037$ ) for SOD activity were observed. As shown in a post hoc analysis, SOD activity was significantly higher at preexercise, postexercise, and recovery in the supplemented group than in the placebo group ( $p < .05$ ).

Table 4 presents changes in the assessed oxidative-stress markers. Similar to SOD levels, a significant main effect for GliSODin ( $p < .001$ ) on serum CRP concentrations was observed. Levels of CRP before and after exercise and after 24-hr restitution in the supplemented group were significantly lower than in the placebo group.

TBARS levels (Table 4) were not significantly affected by the supplementation (main effect  $p = .257$ ) but changed significantly after the exercise test ( $p < .001$ ). In both groups exercise caused a significant increase in TBARS concentrations directly after exercise both before (by 57% in the supplemented group and 63% in the placebo group) and after supplementation (by 59% in the supplemented group and 70% in the placebo group). In both groups TBARS levels remained increased during restitution only before supplementation, whereas a slight, nonsignificant decrease in their concentrations was observed after supplementation.

ANOVA showed no significant interaction effect between supplementation and exercise on plasma CK activity (Table 4). In contrast, a significant main effect of exercise ( $p < .001$ ) on plasma lactate dehydrogenase activity was found. In both groups the postexercise levels of LDH were higher than preexercise both before and after supplementation.

Pearson's correlation analysis revealed high correlation between TBARS concentrations in erythrocytes and blood lactate levels (before supplementation,  $r = .77$ ,  $p < .001$ ; after supplementation,  $r = .61$ ,  $p < .001$ ) and moderate correlation between lactate dehydrogenase levels and TBARS concentrations ( $r = .43$ ,  $p < .05$ ).

## Discussion

The current study examined effects of supplementation with GliSODin on changes in parameters describing the balance between oxidants and antioxidants and on inflammatory-marker levels in professional rowers performing a 2,000-m time trial at the beginning and end of a 6-week preparatory camp between the preparation and competition periods. To the best of our knowledge, it is the first study to investigate the results of supplementation with melon extract on changes in redox parameters induced by

exercise. Despite increasing activity of some antioxidant enzymes (SOD), GliSODin failed to prevent oxidative damage resulting from acute exercise of high intensity in our study population. In contrast, we observed decreased CRP levels after supplementation with GliSODin, which suggests that melon extract may have anti-inflammatory effects in intensively training athletes.

A 2,000-m time trial is a standard test used to assess performance in rowers (Mäestu, Jürimäe, & Jürimäe, 2005). Even though oxygen uptake was not assessed during the test, it involved high-intensity exercise; subjects were to cover the distance in as short a time as possible with all-out effort. High intensity of exercise was confirmed by high maximum lactate levels (Table 2), implying intensity close to maximum oxygen uptake and a high contribution of anaerobic exercise (Messonnier, Freund, Bourdin, Belli, & Lacour, 1997). It is generally accepted that such intense exercise is associated with oxidative stress in tissues and in the blood (Veskoukis et al., 2008). In accordance with these views, we observed significant increases in TBARS levels in erythrocytes after exercise tests performed on both occasions in both study groups (Table 4). Assessment of TBARS is regarded as a general measure of lipid peroxidation (Groussard et al., 2003; Rimbach et al., 1999), and TBARS was used in our study as an oxidative-stress marker. We found a high correlation between TBARS concentrations in erythrocytes and blood lactate levels, which corresponds well with the results of some studies indicating a relationship between intensity of exercise and oxidative stress (Ashton et al., 1998). Similar to our results, Kyparos, Vrabas, Nikolaidis, Riganas, and Kouretas (2009) demonstrated a significant increase in serum TBARS concentrations after a simulation test consisting of a 2,000-m maximal effort on a rowing ergometer in well-trained male rowers during the preseason preparatory training period.

Elevations of serum muscle enzymes (CK and LDH) are universally accepted as a marker of tissue damage (Kanter, Lesmes, Kaminsky, La Ham-Saeger, & Nequin, 1988; Petibois, Cazorla, Poortmans, & Déléris, 2002). We observed an increase in LDH blood levels directly after the 2,000-m time trial before and after intervention in both groups of rowers (Table 4), which indicates exercise-induced damage to muscle cell membrane. CK activity changed in a similar pattern, although the differences were not significant, probably because of high standard deviation of the mean (Table 4). High dispersion of CK values in athletes subjected to strenuous exercise has previously been observed by several investigators (Schröder, Navarro, Mora, Galiano, & Tramullas, 2001; Teixeira et al., 2009). Lactate dehydrogenase levels moderately correlated with TBARS concentrations in our study. Kanter et al. suggested that damage to muscle cell membrane may have been related to an exercise-induced lipid peroxidation. Results of more recent studies indicate that muscle-damaging exercise induces lipid peroxidation in human blood (Bailey et al., 2004; Groussard et al., 2003; Rimbach et al., 1999). However, elevation in lipid-peroxidation markers in most of these

**Table 4 Changes in Free-Radical Production and Muscle-Damage Markers During Exercise Tests Before and After Supplementation (M ± SD)**

Parameter	Before Supplementation			After Supplementation			Exercise × GliSODin
	Preexercise	Postexercise	Recovery	Preexercise	Postexercise	Recovery	
CRP (mg/L)							
Sup	0.46 ± 0.31	0.49 ± 0.39	0.50 ± 0.37	0.18 ± 0.08*	0.21 ± 0.10*	0.23 ± 0.11*	<i>p</i> = .915
Pla	0.28 ± 0.26	0.54 ± 0.50	0.41 ± 0.39	0.61 ± 0.56	0.68 ± 0.62	0.66 ± 0.57	<i>p</i> < .001
TBARS (μmol/g Hb)							
Sup	1.77 ± 0.56	2.78 ± 1.11†	3.08 ± 0.89†	1.19 ± 0.31	1.89 ± 0.24†	1.71 ± 0.62	<i>p</i> < .001
Pla	1.51 ± 0.69	2.46 ± 0.69†	2.61 ± 0.78†	1.45 ± 0.59	2.47 ± 0.75†	1.77 ± 0.58	<i>p</i> = .257
CK (U/L)							
Sup	166 ± 86	204 ± 100	167 ± 79	75 ± 41	92 ± 46	146 ± 91	<i>p</i> = .150
Pla	210 ± 163	259 ± 177	224 ± 120	110 ± 88	130 ± 117	213 ± 132	<i>p</i> = .261
LDH (U/L)							
Sup	153 ± 68	242 ± 64†	195 ± 49	182 ± 51	262 ± 50†	185 ± 41	<i>p</i> < .001
Pla	128 ± 45	270 ± 43†	209 ± 37†	192 ± 78	279 ± 23†	205 ± 23	<i>p</i> = .515
							<i>p</i> = .687

Note. CRP = C-reactive protein; Sup = supplemented group; Pla = placebo group; TBARS = thiobarbituric-acid-reactive substances; CK = creatine kinase; LDH = lactate dehydrogenase.

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

studies was observed within hours or days of recovery period, not immediately after the exercise as it was in our study (Table 4).

We hypothesized that supplementation with GliSODin, containing vegetable SOD coupled with gliadin biopolymer, would enhance the antioxidant system and prevent exercise-induced oxidative stress and muscle-membrane injury. We observed significant increase in SOD activity in erythrocytes at rest and after exercise in rowers receiving this preparation (Table 3). This suggests that GliSODin is bioavailable when taken orally and can affect the intracellular antioxidant system. In contrast, supplementation with GliSODin had no effect on other assessed antioxidants (GPx, TAC) or TBARS levels, either at rest or postexercise, and failed to prevent muscle injury assessed with CK and LDH (Table 3 and 4). Studies on the effects of GliSODin or gliadin supplementation on oxidative-stress markers and associated damages in laboratory animals and humans are sparse, and their results are equivocal. Vouldoukis et al. (2004) observed raised activity of antioxidant enzymes (SOD, GPx, and catalase) in blood and liver and improved antioxidant status of plasma in mice after 28-day supplementation with standardized melon extract coupled with gliadin (1 mg/day). In contrast, Muth et al. (2004) reported decreased GPx activity in divers subjected to 60-min hyperbaria (2.5 atm) after 2 weeks of supplementation with GliSODin at a dose of 1,000 IU daily. Supplementation with melon extract in their study resulted in reduced oxidative damage to DNA in comparison with controls, without affecting other oxidative-stress markers and antioxidant parameters (SOD and catalase, and malondialdehyde concentrations in red blood cells). Similarly, Kick et al. (2007) observed that GliSODin limited DNA oxidative damage but did not influence markers of lipid peroxidation in animals after 30 min of thoracic aortic cross-clamping and 4 hr of reperfusion.

Data on the effects of intense exercise on antioxidant enzyme activity and nonenzymatic antioxidants are inconsistent, depending on the type of exercise (aerobic vs. anaerobic), type of antioxidant assessed, and population under study (Finaud, Lac, & Filaire, 2006). We observed significant changes in the antioxidant system in the blood after the 2,000-m test performed after the supplementation period (that is, at the end of the preparatory camp) but not at the beginning of the study. GPx activity in erythrocytes increased directly after the ergometry in both study groups, and total antioxidant capacity of plasma was augmented in the placebo group (Table 3). Increased GPx activity observed in both groups after the preparatory camp was probably an adaptive response to training. In accordance with our findings, Teixeira et al. (2009) observed higher GPx activity in the whole blood of kayakers during the preparation period in comparison with the competition period, when bouts of extremely intensive exercise might have weakened their antioxidant defense system. Increased GPx activity was also found in other studies performed on athletes involved in various sports (Hubner-Wozniak, Lutoslawska, Sendekci,

Sitkowski, & Borkowski, 1996; Tessier, Margaritis, Richard, Moynot, & Marconnet, 1995). Moreover, in the study of Hubner-Wozniak et al. the increase in GPx activity correlated with maximum oxygen uptake.

TAC is a global marker encompassing different circulating antioxidants of plasma. It is thought that uric acid, an end product of purine metabolism, is the most important plasma antioxidant *in vivo* (Kaur & Halliwell, 1990). According to Wayner, Burton, Ingold, Barclay, and Locke (1987), uric acid determines about 35–65% of TAC. It has been demonstrated in experimental studies that it may be redistributed from plasma to skeletal muscle to be used as an intracellular antioxidant, particularly during exercise-induced oxidative stress (Hellsten-Westing, Kaijser, Ekblom, & Sjödin, 1994). Schneider, Barp, Ribeiro, Belló-Klein, and Oliveira (2005), in a study investigating effects of low-, medium- and high-intensity running on antioxidant potential, demonstrated that in contrast to untrained subjects, only high-intensity exercise (above anaerobic threshold) was associated with a significant increase in TAC in triathletes. A significant increase of TAC (by 25%) was also reported by Skenderi et al. (2008) in athletes after prolonged, exhaustive exercise (246-km run). In contrast, Kyparos et al. (2009) observed only a slight increase in TAC (9%) after a 2,000-m rowing-ergometer race in well-trained athletes. It seems probable that postexercise changes in TAC depend not only on the intensity and duration of exercise but also on the TAC level at rest. Perhaps this is why TAC levels did not increase after the 2,000-m test in the supplemented group in our study, because their values at rest were slightly (nonsignificantly) higher than in the subjects receiving the placebo (Table 3).

In addition to increases in GPx activity and TAC directly after the 2,000-m time trial performed after the preparatory camp, TBARS levels after the 24-hr recovery period did not differ from the preexercise values (Table 4). In contrast, TBARS concentrations remained elevated 24 hr after the ergometry performed at the beginning of the study (Table 4). Lower TBARS levels 24 hr after exercise indicate more efficient detoxification and metabolism of the substance (Argüelles, García, Maldonado, Machado, & Ayala, 2004). Favorable changes in TBARS levels (Table 4) and antioxidant parameters (Table 3) after the 2,000-m time trial performed at the end of the study most probably resulted from better adaptation of the athletes to strenuous exercise at the beginning of the competition period, as a direct result of training.

Exercise-induced changes in redox parameters were similar in both supplemented and placebo groups, and we observed no significant interaction between exercise and supplementation (Table 3 and 4). These findings indicate that changes in redox parameters in response to exercise at the end of the study cannot be attributed to supplementation with melon extract. Intense exercise is associated with hemolysis leading to an increase in plasma levels of iron ions, which act as a catalyst in free-radical reactions (Córdova Martínez, Villa, Aguiló, Tur, & Pons, 2006; Zunquin et al., 2006). Chelation of transition-metal ions

(including Fe<sup>2+</sup>) removes the substrate in the Fenton reaction and prevents formation of highly reactive hydroxyl radical, which initializes peroxidation of polyunsaturated fatty acids in erythrocyte membrane. It was recently shown that supplementation with iron impairs oxidative status in intensively exercising subjects. Casein, being an iron-chelating agent, diminished TBARS levels in liposomes (Zunquin et al., 2006). The failure of GliSODin to prevent exercise-induced lipid peroxidation is probably a result of the fact that the melon preparation has no chelating properties.

It is suggested that damage to muscle cells caused by free radicals formed during exercise may initialize inflammation (Vassilakopoulos et al., 2003). Fatouros et al. (2006) investigated changes in levels of an inflammatory marker—CRP—after intensive exercise in men (8 resistance multijoint exercises). They found that increase in exercise volume was associated with the highest rise (400%) in CRP levels, and increase in exercise intensity augmented CRP levels to a lesser degree (300%). They also noted that the rise in CRP levels after intensive exercise may be time lagged. Park, Park, Kim, and Kwak (2008) observed an increase in plasma CRP levels 7 days after exercise in triathletes. Finally, Miles, Walker, Conant, Hogan, and Kidd (2006) reported a rise in CRP levels 4 hr after a 32-km mountain trial race; CRP concentrations further increased after 24-hr restitution. In contrast, we observed no changes in CRP levels after the 2,000-m test performed both before and at the end of the preparatory camp. Despite its high intensity, the exercise's duration was probably not long enough to influence CRP concentration. Meyer, Gabriel, Rätz, Müller, and Kindermann (2001) showed that repeated short anaerobic bouts of cycling led to an acute-phase response that was more pronounced than after a single bout.

Six weeks of supplementation with the melon preparation resulted in a decreased level of CRP in the supplemented group in comparison with the athletes receiving the placebo (Table 4). This effect cannot be attributed to limited muscle-cell damage, because GliSODin failed to alter LDH or CPK activity. The results of a few studies suggest that melon extract may have direct anti-inflammatory properties (Dugas et al., 2003; Marikovsky, Ziv, Nevo, Harris-Cerruti, & Mahler, 2003; Okada et al. 2006; Vouldoukis et al., 2003). Vouldoukis et al. (2004) observed that melon extract decreased formation of tumor necrosis factor alpha and stimulated production of anti-inflammatory cytokines such as interleukin 10. They also found a significant, inverse correlation between these parameters. Anti-inflammatory potential of melon extract may be of particular interest in sport activities involving prolonged, intense exercise that is associated with systemic inflammatory response (Fatouros et al., 2006; Miles et al., 2006; Park et al., 2008).

The study has several limitations. We did not assess maximum oxygen consumption, so we could not determine the intensity of exercise during the 2,000-m test as a percentage thereof, and we cannot be certain the effort was really maximal. For the same reason we could not

precisely determine training intensity; instead, we classified the training loads based on lactate threshold.

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