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ORIGINAL ARTICLE

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Strenuous endurance training in humans reduces oxidative stress following exhausting exercise

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Abstract The aim of this study was to evaluate whether high-intensity endurance training would alleviate exercise-induced oxidative stress. Nine untrained male subjects (aged 19–21 years) participated in a 12-week training programme, and performed an acute period of exhausting exercise on a cycle ergometer before and after training. The training programme consisted of running at 80% maximal exercise heart rate for 60 min · day^{-1}, 5 days · week^{-1} for 12 weeks. Blood samples were collected at rest and immediately after exhausting exercise for measurements of indices of oxidative stress, and antioxidant enzyme activities [superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT)] in the erythrocytes. Maximal oxygen uptake ($\dot{V}O_{2max}$) increased significantly ($P < 0.001$) after training, indicating an improvement in aerobic capacity. A period of exhausting exercise caused an increase ($P < 0.01$) in the ability to produce neutrophil superoxide anion ($O_2^{\bullet -}$) both before and after endurance training, but the magnitude of the increase was smaller after training ($P < 0.05$). There was a significant increase in lipid peroxidation in the erythrocyte membrane, but not in oxidative protein, after exhausting exercise, however training attenuated this effect. At rest, SOD and GPX activities were increased after training. However, there was no evidence that exhausting exercise enhanced the levels of any antioxidant enzyme activity. The CAT activity was unchanged either by training or by exhausting exercise. These results indicate that high-intensity endurance training can elevate antioxidant enzyme activities in erythrocytes, and decrease neutrophil $O_2^{\bullet -}$ production in response to exhausting exercise. Furthermore, this up-regulation in antioxidant defences was accompanied by a reduction in exercise-induced lipid peroxidation in erythrocyte membrane.

Key words Superoxide anion · Endurance training · Oxidative damage · Antioxidant enzyme · Erythrocyte

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Introduction

During strenuous exercise, the metabolic rate in the skeletal muscle is raised up to 100 times above resting levels, reflected by markedly increased oxygen consumption. This increase in oxygen consumption can lead to an elevation of superoxide anion ($O_2^{\bullet -}$) production in the mitochondria (Davies et al. 1982; Jenkins 1988). Subsequent reactions give rise to other reactive oxygen species (ROS), i.e. hydrogen peroxide and extremely reactive hydroxyl radical. The ROS has been shown to induce damage in all cellular macromolecules, such as lipids, proteins, and DNA (Sen 1995). Therefore, an increase in the generation of ROS during exercise has been considered to be an oxidative stress (Davies et al. 1982). Even moderate exercise may increase ROS production exceeding the capacity of antioxidant defences (Alessio 1993; Ji 1993). We showed that maximal exer-

cise induced a larger increase in lipid peroxidation compared to moderate exercise (Toshinai et al. 1998). In addition, exhausting exercise has been shown to cause a change in glutathione redox status in human blood inducing an oxidative stress (Sastre et al. 1992).

Erythrocytes are susceptible to oxidative damage as a result of the high polyunsaturated fatty acid content of their membrane and the high cellular concentrations of oxygen and haemoglobin (Hb), a potentially powerful promoter of oxidative processes (Clemens and Waller 1987). Furthermore, neutrophils are the main sources of extracellular ROS production in the blood. Neutrophils have been shown to generate superoxide by activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase located on the plasma membrane (Pyne 1994). The NADPH oxidase system has been shown to be activated in response to various stimuli that can be provoked by strenuous exercise (Suzuki et al. 1996). This is especially important when superoxide is involved in the initial oxidative injury since superoxide activates a chemotactic factor that attracts polymorphonuclear neutrophils. While this is a desirable reaction under most circumstances, it may also provide a secondary source of ROS production causing further tissue injury, including erythrocytes, because erythrocytes in blood exist around neutrophils, the number of the former being several times as many as that of the latter. However, it is still unclear whether superoxide production by neutrophils is relevant to erythrocyte oxidative stress. Erythrocytes are exposed to ROS that are constantly generated from both internal and external sources even under normal conditions, and they may be targeted for oxidative damage during exercise. However, erythrocytes contain many antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT), as well as nonenzymatic antioxidants such as vitamin E, vitamin C, glutathione and ceruloplasmin. These findings suggest that erythrocytes maintain a high antioxidant defence capacity. In a previous study, we have shown that 10 weeks of training increases CAT and total glutathione reductase activities in erythrocytes (Ohno et al. 1988). However, it is unclear whether a chronic adaptation of the erythrocytes' antioxidant enzymes can reduce oxidative damage following exhausting exercise. It is also unclear whether neutrophil $O_2^{\bullet -}$ production is altered by acute exercise or chronic training.

Thus, the aim of this study was to investigate whether high intensity endurance training reduces exercise-induced oxidative stress in human erythrocytes, and if so whether this reduction is causes by induction of antioxidant enzymes, and/or attenuation of neutrophil oxidant production.

Methods

Subjects

Nine healthy male subjects participated in the study, aged 19–21 years (Table 1). None was involved in a regular training programme before the study. The subjects were informed of the procedures involved and any possible risks and discomfort associated with the experiment before giving written consent. This trial was conducted following the guidelines of the Helsinki Declaration of 1975.

Table 1 Effects of 12 weeks of training on the physical characteristics of the subjects ($n = 9$)

	Before		After	
	Mean	SEM	Mean	SEM
Age (years)	19.4	0.2		
Height (cm)	174	1	174	1
Body mass (kg)	70.5	2.6	70.4	2.7
Body mass index	23.4	0.6	23.3	0.7
Body fat (%)	15.3	0.7	15.5	0.7
Maximal O_2 uptake (ml kg^{-1} min^{-1})	44.9	1.5	49.7***	1.6

***: $P < 0.001$ Compared to Before

Experiment protocol

Before and after a 12 week training programme, the subjects performed an incremental exercise test until exhaustion using a cycle ergometer (Monark, Stockholm, Sweden) at a constant pedal speed of 60 rpm with stepwise increments of 15 W every minute after a warm-up at 15 W for 3 min. The gas exchange during exercise was analysed from expired gases using an Oxycongamma (Mijnhardt, Bunnik, Netherlands). Heart rate (HR) was continuously monitored during the test. The endurance training programme consisted of running at 80% maximal heart rate (HR_{max}) for 60 min · day^{-1}, 5 days · $week^{-1}$ for 12 weeks. Exercise intensity was adjusted as the subject's aerobic capacity increased. Subjects measured their own HR during running exercise under the supervision of our technicians. Percentage body fat was estimated by measuring skinfold thickness. A Lange caliper (Eiyoken-type, Meikosha, Nagoya, Japan) was used to quantify skinfold thickness at the two sites (biceps and subscapular), and percentage body fat was calculated using the equation of Brožek et al. (1963).

Blood sampling and antioxidant enzymes assay

Heparinized blood samples were obtained from an antecubital vein at rest and immediately after the exhausting exercise. The blood (7 ml) was centrifuged (750*g*, 4 °C, 10 min), and erythrocytes were separated. Erythrocyte fractions were resuspended and washed three times with cold isotonic saline solution. Washed erythrocytes were stored at −80 °C until analysis.

For measurement of the antioxidant enzyme activities, the erythrocytes were haemolysed in 0.05% β-mercaptoethanol with 10% ethylenediaminetetra-acetic acid. The erythrocyte SOD [Enzyme Commission no. (EC) 1.15. 1.1] activity was determined using the method of Crapo et al. (1978). The GPX (EC 1.11.1.9) activity was measured using the spectrophotometric assay described by Tappel (1978). The CAT (EC 1.11.1.6) activity was assayed using the method of Aebi (1984). All enzyme activities were expressed relative to the Hb concentration.

Neutrophil isolation

Neutrophils were isolated from the peripheral blood by a one-step centrifugal technique. Heparinized blood (5 ml) was decanted on to the top of an equal volume of a Polymorphprep (NYCOMED, Oslo, Norway) which separated monocytes and neutrophils, and was then centrifuged at 500*g* for 30 min. The polymorphonuclear cell layer was harvested using a Pasteur pipette, and its fraction was diluted by adding one volume of 0.45% NaCl solution to restore normal

osmolality. The cell suspension was washed twice with Hank's balanced salt solution (HBSS) by centrifugation at 400*g* at 4 °C for 10 min. The cells were suspended to 1×10^7 cells ml^{-1} in HBSS and kept at 0 °C for no longer than 2 h prior to use.

Assay for the ability of $O_2^{\bullet-}$ production by neutrophils

The ability of neutrophils to produce $O_2^{\bullet-}$ was assessed by the intensity of 2-methyl-6(p-methoxyphenyl)-3,7-dihydroimidazo[1,2-a]pyrazine-3-one (MCLA; Tokyo Kasei, Tokyo, Japan)-dependent chemiluminescence according to the method of Nakano et al. (1986) with a slight modification. Zymosan (OZ; Sigma, St. Louis, Mo., USA) was opsonized in human serum at 37 °C for 30 min, and opsonized OZ (final concentration: 20 mg ml^{-1}) was stored at −80 °C until used, and used once after thawing. Some MCLA was dissolved in distilled water and stored at −80 °C until needed. The reaction mixture contained 1×10^5 neutrophils, 100 µl of OZ (2 mg in 2 ml), and 1 µM of MCLA in 2.0 ml of continuously stirred HBSS. The intensity of luminescence was monitored using the luminescence reader BLR-301 (Aloka Co., Tokyo, Japan) set at 37 °C. The reactions were started with the addition of MCLA and OZ. Some Cu,Zn-SOD (Wako, Tokyo, Japan) was added to stop the reaction. The maximal intensity of OZ-stimulated neutrophils was measured as a peak, and the reaction without OZ was monitored as a control. The difference between the peak and control values was then defined as the ability of neutrophils to produce $O_2^{\bullet-}$. The data were expressed as percentages of each level at rest before training.

Oxidative damage of the erythrocyte membrane

The erythrocyte membrane was used to measure oxidative damage such as lipid peroxidation and protein oxidation, and prepared by centrifugation (20,000*g*, 4 °C, 30 min) three times with hypotonic solution. Lipid peroxidation was estimated using the measurement of thiobarbituric acid-reactive substance (TBARS) level according to the method of Ohkawa et al. (1979). Reactive carbonyl derivative (RCD) contents as a marker of protein oxidation was measured using the method of Levine et al. (1990).

Statistical analysis

The statistical significance of the data was assessed by a two-way analysis of variance (ANOVA) with repeated measure and Bonferroni post hoc comparison. When applicable, the paired Student's *t*-test was used. A $P < 0.05$ level of significance was used.

Results

Physical characteristics

As shown in Table 1, $\dot{V}O_{2max}$ of the subjects increased significantly (10.7%, $P < 0.001$) after 12 weeks of training, indicating an improvement in aerobic capacity. The time to reach exhaustion increased significantly after endurance training from a mean of 14.4 (SEM 0.4) min to a mean of 17.8 (SEM 0.3) min, and the mean exercise intensity at the end of the exercise test also increased from 202 (SEM 6) W to 258 (SEM 6) W. There were no changes in the other physical characteristics after training.

Neutrophil $O_2^{\bullet-}$ production

Exhausting exercise caused an increase in the ability of the neutrophils to produce $O_2^{\bullet-}$ ($P < 0.01$) regardless of the running training. However, the magnitude of the increase was smaller ($P < 0.05$) after training (Fig. 1).

Oxidative damage of the erythrocyte membrane

The TBARS levels as a index of lipid peroxidation were increased ($P < 0.05$) in response to exhausting exercise both before and after training. However, endurance training decreased the amount of exercise-induced lipid peroxidation ($P < 0.05$; Fig. 2). Meanwhile, RCD levels, markers of oxidized protein, were unchanged either by exhausting exercise or by training (Fig. 3).

Antioxidant enzyme activities

The activities of SOD and GPX increased by 17.1% ($P < 0.01$) and 11.5% ($P < 0.05$), respectively, after

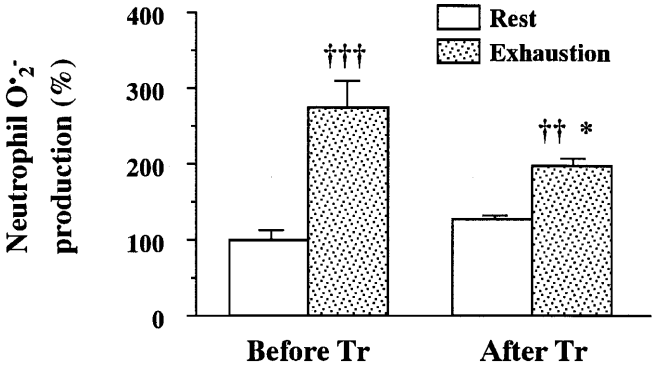

Fig. 1 Effects of endurance training (*Tr*) on the ability of neutrophils to produce superoxide anion ($O_2^{\bullet-}$). The data are expressed as percentages of the concentrations measured at rest before training. Values are means and SEM. Significantly different from Rest ††: $P < 0.01$, †††: $P < 0.001$, respectively, significantly different from Before *Tr* *: $P < 0.05$

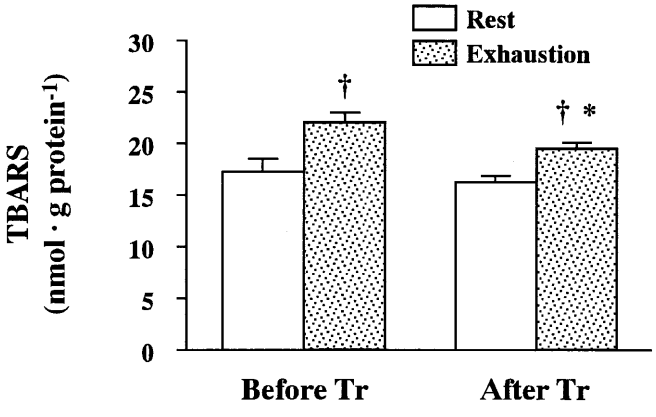

Fig. 2 Effects of endurance training (*Tr*) on exercise-induced lipid peroxidation as measured by thiobarbituric acid reactive substances (*TBARS*) in the erythrocyte membrane. Values are means and SEM. Significantly different from Rest †: $P < 0.05$, significantly different from Before *Tr* *: $P < 0.05$

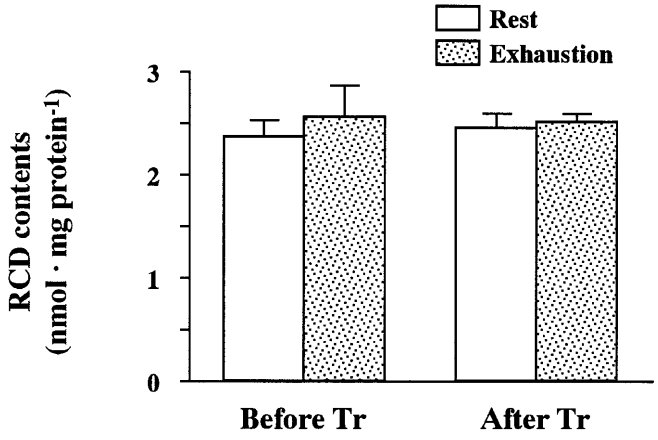

Fig. 3 Effects of endurance training (*Tr*) on exercise-induced oxidative protein assessed by reactive carbonyl derivative (*RCD*) contents in the erythrocyte membrane. Values are means and SEM

12 weeks of intense training (Table 2). On the other hand, these antioxidant enzyme activities were unaffected by the period of acute maximal exercise. No change in CAT activity was observed with either maximal exercise or endurance training.

Discussion

The results of the present study show that $\dot{V}O_{2max}$ and the time to reach exhaustion increased by 10.7% and 23.6%, respectively, after the training programme, indicating an improvement in aerobic capacity. However, body composition, such as body fat, did not change substantially in spite of strenuous training. This result may be explained by the low percentage body fat of the subjects prior to training. A significant change in body composition usually takes a longer period of training to become manifest.

Exhausting exercise increased the ability of neutrophils to produce $O_2^{\bullet-}$ regardless of training; however, the intense running training reduced exercise-induced $O_2^{\bullet-}$ production. We have previously shown that the ability of neutrophils to generate $O_2^{\bullet-}$ was markedly increased in untrained rats after endurance exercise (Ohishi et al. 1997). The mechanisms which induce the priming of neutrophils is not clear, but it may be related to various changes such as in cytosolic Ca^{2+} concentration and the number of the plasma membrane receptors, which are thought to be influenced by neuroendocrine hormones, cytokines and chemoattractants (Pyne 1994). Ashton et al. (1998) demonstrated that exhausting exercise caused an approximately threefold increase in free radical concentration in the venous circulation of humans and increased serum lipid peroxidation concentrations. Circulating hormones such as catecholamines have been shown to rise during exercise (Kindermann et al. 1982). Furthermore, catecholamines increase the circulating leucocyte counts by mobilizing leucocytes from marginated pools. Van Eeden et al. (1999) suggested that circulating hormones generated during exercise are unlikely to be responsible for the increase in neutrophil activation, because the neutrophils mobilized during exercise are older compared to originally circulating neutrophils. However, there is evidence that the exercise-mobilized older neutrophils produce more oxygen radicals than younger ones when stimulated (Tanji-Matsuba et al. 1998). Moreover, acute exercise has been shown to increase the expression of complement 3bi receptors on the granulocyte plasma membrane (Hashimoto et al. 1996). Therefore, our results may have been due to a down regulation of the sensitivity of the neutrophils to factors that stimulate superoxide generation, such as receptor density or a decrease in catecholamine release during exercise, or both, after training.

The ROS rapidly react with polyunsaturated fatty acids in the cell membranes, proteins, and other cell components. Therefore, these radicals may attack lipids and proteins in the erythrocyte, because of the high content of haem-iron and polyunsaturated fatty acids. In this study, the levels of lipid peroxidation in the erythrocyte membrane increased after maximal exercise irrespective of training status. However, the increases in these levels were reduced by training. Endurance training increased oxygen consumption during an incremental exercise test but decreased lipid peroxidation following exhausting exercise compared with the pre-training. Such interesting results on erythrocytes might be related to the balance of free radical generation and scavenging enzymes such as SOD, CAT and GPX. Furthermore, TBARS levels in erythrocytes showed a similar pattern to the superoxide production by neutrophils after the training programme. Therefore, the oxidative stress in erythrocytes might be attributable, in part, to ROS production by neutrophils. The results of this study differ from the finding by Koz et al. (1992) in

Table 2 Erythrocyte antioxidant enzyme activities following exhausting exercise before and after running training ($n = 9$). *SOD* Superoxide dismutase, *GPX* glutathione peroxidase, *CAT* catalase, *Hb* haemoglobin

	Before				After			
	Rest		Exhaustion		Rest		Exhaustion	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
SOD (U g Hb^{-1})	1,518	41	1,606	29	1,778*	70	1,791	38
GPX (U g Hb^{-1})	31.7	0.4	32.7	1.1	35.3*	1.1	34.0	0.8
CAT (*K* g Hb^{-1})	31.8	0.4	31.2	0.2	31.4	0.2	31.3	0.3

*: $P < 0.05$ Compared to Before

that the erythrocyte malondialdehyde levels in rats did not change following acute swimming exercise. These conflicting results may be attributable to methodological differences, especially in the use of nonspecific assays to detect lipid peroxidation and variations in exercise duration and intensity. In the present study, the levels of oxidative protein were unaffected by either maximal exercise or training. This finding was consistent with several studies showing that protein carbonyl concentration in rat skeletal muscles after an exhausting bout of endurance exercise was not significantly elevated (Bejma and Ji 1999; Reznick et al. 1992). Radák et al. (1997) suggested that lipid peroxidation and protein oxidation may involve different mechanisms in vivo. For example, Leeuweuburgh et al. (1999) showed that intense exercise can enhance protein tyrosine and phenylalanine oxidation due to increased OH^- formation in rats. The RCD assay may not be sensitive enough to detect these damaging effects.

The up-regulations of erythrocyte SOD and GPX activities were induced by 12 weeks of endurance training. However, these activities of antioxidant enzymes did not change after exhausting exercise. These results were consistent with the findings that erythrocyte antioxidant enzyme activities were not affected in response to acute exercise in humans (Duthie et al. 1990; Ohno et al. 1986), but can be induced by chronic exercise training (Ohno et al. 1988).

At rest, cells have sufficient non-enzymatic and enzymatic antioxidant systems to remove and prevent harmful effects of ROS (Jenkins 1988). However, Smith et al. (1995) suggested that submaximal exercise might cause significant changes in erythrocyte susceptibility to oxidative stress, and that these responses may account for the increase in erythrocyte turnover found in athletes undertaking strenuous training. Margaritis et al. (1997) suggested that the magnitude of enhancement of the antioxidant defence system depends on the amount of training. Strenuous long duration exercise and exhausting training overwhelm the capacity to detoxify ROS, producing oxidative stress (Marzatico et al. 1997). Thus, endurance training can elevate the erythrocyte activities of free radicals-scavenging enzymes.

We conclude that intense endurance training can elevate antioxidant enzyme activities in erythrocytes as well as aerobic capacity. In addition, it decreases the ability of neutrophils to produce $O_2^{\bullet -}$ following exhausting exercise. Therefore, this up-regulation of the antioxidant defence system would result in a reduction of exercise-induced lipid peroxidation in the erythrocyte membrane. Thus, such a 12 week running training programme may be an effective strategy to up-regulate the antioxidant defence system in the erythrocytes, which increases their resistance to subsequent oxidative stress.

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References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105: 121–126

Alessio HM (1993) Exercise-induced oxidative stress. Med Sci Sports Exerc 25: 218–224

Ashton T, Rowlands CC, Jones E, Young IS, Jackson SK, Davies B, Peters JR (1998) Electron spin resonance spectroscopic detection of oxygen-centred radicals in human serum following exhaustive exercise. Eur J Appl Physiol 77: 498–502

Bejma J, Ji LL (1999) Aging and acute exercise enhance free radical generation in rat skeletal muscle. J Appl Physiol 87: 465–470

Brožek J, Grande F, Anderson JT, Keys A (1963) Densitometric analysis of body composition. Review of some quantitaive assumptions. Ann NY Acad Sci 110: 113–140

Clemens MR, Waller HD (1987) Lipid peroxidation in erythrocytes. Chem Phys Lipids 45: 251–268

Crapo JD, McCord JM, Fridovich I (1978) Preparation and assay of superoxide dismutases. Methods Enzymol 53: 382–389

Davies KJ, Quintanilha AT, Brooks GA, Packer L (1982) Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 107: 1198–1205

Duthie GG, Robertson JD, Maughan RJ, Morrice PC (1990) Blood antioxidant status and erythrocyte lipid peroxidation following distance running. Arch Biochem Biophys 282: 78–83

Hashimoto Y, Suzuki D, Hamada K, Okada H, Nagao N (1996) Changes of expression of complement 3bi receptors on granulocytes after physical exercise in rats. J Sports Med Phys Fitness 36: 275–280

Jenkins RR (1988) Free radical chemistry: relationship to exercise. Sports Med 5: 156–170

Ji LL (1993) Antioxidant enzyme response to exercise and aging. Med Sci Sports Exerc 25: 225–231

Kindermann W, Schnabel A, Schmitt WM, Biro G, Cassens J, Weber F (1982) Catecholamines, growth hormone, cortisol, insulin, and sex hormones in anaerobic and aerobic exercise. Eur J Appl Physiol 49: 389–399

Koz M, Erbas D, Bilgihan A, Aricioglu A (1992) Effects of acute swimming exercise on muscle and erythrocyte malondialdehyde, serum myoglobin, and plasma ascorbic acid concentrations. Can J Physiol Pharmacol 70: 1392–1395

Leeuwenburgh C, Hansen PA, Holloszy JO, Heinecke JW (1999) Hydroxyl radical generation during exercise increases mitochondrial protein oxidation and levels of urinary dityrosine. Free Radic Biol Med 27: 186–192

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER (1990) Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 186: 464–478

Margaritis I, Tessier F, Richard M-J, Marconnet P (1997) No evidence of oxidative stress after a triathlon race in highly trained competitors. Int J Sports Med 18: 186–190

Marzatico F, Pansarasa O, Bertorelli L, Somenzini L, Valle GD (1997) Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. J Sports Med Phys Fitness 37: 235–239

Nakano M, Sugiura K, Ushijima Y, Goto T (1986) Chemiluminescence probe with Cypridina luciferin analog, 2-methyl-6-phenyl-3, 7-dihydroimidazo[1,2-a]pyrazin-3-one, for estimating the ability of human granulocytes to generate O_2^-. Anal Biochem 159: 363–369

Oh-ishi S, Kizaki T, Ookawara T, Sakurai T, Izawa T, Nagata N, Ohno H (1997) Endurance training improves the resistance of rat diaphragm to exercise-induced oxidative stress. Am J Respir Crit Care Med 156: 1579–1585

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351–358

Ohno H, Sato Y, Yamashita K, Doi R, Arai K, Kondo T, Taniguchi N (1986) The effect of brief physical exercise on free

radical scavenging enzyme systems in human red blood cells. Can J Physiol Pharmacol 64: 1263–1265

Ohno H, Yahata T, Sato Y, Yamamura K, Taniguchi N (1988) Physical training and fasting erythrocyte activities of free radical scavenging enzyme systems in sedentary men. Eur J Appl Physiol 57: 173–176

Pyne DB (1994) Regulation of neutrophil function during exercise. Sports Med 17: 245–258

Radák Z, Asano K, Lee KC, Ohno H, Nakamura A, Nakamoto H, Goto S (1997) High altitude training increases reactive carbonyl derivatives but not lipid peroxidation in skeletal muscle of rat. Free Radic Biol Med 22: 1109–1114

Reznick AZ, Witt E, Matsumoto M, Packer L (1992) Vitamin E inhibits protein oxidation in skeletal muscle of resting and exercised rats. Biochem Biophys Res Commun 189: 801–806

Sastre J, Asensi M, Gasco E, Pallardo FV, Ferrero JA, Furukawa T, Viña J (1992) Exhaustive physical exercise causes oxidation of glutathione status in blood: prevention by antioxidant administration. Am J Physiol 263: R992–R995

Sen CK (1995) Oxidants and antioxidants in exercise. J Appl Physiol 79: 675–686

Smith JA, Kolbuch-Braddon M, Gillam I, Telford RD, Weidemann MJ (1995) Changes in the susceptibility of red blood cells to oxidative and osmotic stress following submaximal exercise. Eur J Appl Physiol 70: 427–436

Suzuki K, Sato H, Kikuchi T, Abe T, Nakaji S, Sugawara K, Totsuka M, Sato K, Yamaya K (1996) Capacity of circulating neutrophils to produce reactive oxygen species after exhaustive exercise. J Appl Physiol 81: 1213–1222

Tanji-Matsuba K, Eeden SF van, Saito Y, Okazawa M, Klut ME, Hayashi S, Hogg JC (1998) Functional changes in aging polymorphonuclear leukocytes. Circulation 97: 91–98

Tappel AL (1978) Glutathione peroxidase and hydroperoxides. Methods Enzymol 52: 506–513

Toshinai K, Ohno H, Bae SY, Iwashita T, Koseki S, Haga S (1998) Effect of different intensity and duration of exercise with the same total oxygen uptake on lipid peroxidation and antioxidant enzyme levels in human plasma. Adv Exerc Sports Physiol 4: 65–70

Van Eeden SF, Granton J, Hards JM, Moore B, Hogg JC (1999) Expression of the cell adhesion molecules on leukocytes that demarginate during acute maximal exercise. J Appl Physiol 86: 970–976