Hiromi Miyazaki · Shuji Oh-ishi · Tomomi Ookawara
Takako Kizaki · Koji Toshinai · Sung Ha
Shukoh Haga · Li Li Ji · Hideki Ohno

Strenuous endurance training in humans reduces oxidative stress following exhausting exercise

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 (VO₂max) 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 (O₂⁻) 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 O₂⁻ 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

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 (O₂⁻) 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-
Exercise 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 exhaustive exercise. It is also unclear whether neutrophil O₂⁻ 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 caused 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 pro-

| 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 | 174  | 1    |
| 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 O₂⁻ 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 pedaling 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 Oxycon gamma (Mijnhardt, Bunnik, Netherlands). Heart rate (HR) was continuously monitored during the test. The endurance training programme consisted of running at 80% maximal heart rate (HRmax) 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 Brozek 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 (750g, 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 Polymorph Prep (NYCOMED, Oslo, Norway) which separated monocytes and neutrophils, and was then centrifuged at 500g 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