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The Effects of Short-Term Training on Platelet Functions and Total Antioxidant Capacity in Rats

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Summary

The purpose of the present study was to investigate the effect of short-term endurance training on plasma total antioxidant status (TAS) and on *in vitro* platelet aggregation and ATP release. Blood samples were collected from the abdominal aorta of rats following short-term treadmill exercise (25 m/min, 0 % grade, 30 min) for three consecutive days, as well as in non-exercised control group. Platelet aggregation and platelet ATP release were evaluated by impedance and bioluminescence techniques, respectively. Plasma TAS was measured spectrophotometrically. Plasma TAS was higher and ADP-induced platelet ATP release was lower in the short-term training group with respect to the control group ($p<0.001$). Significant negative correlation ($r = -0.56$, $p<0.05$) was found between plasma TAS and ADP-induced platelet ATP release. Neither ADP- and collagen-induced maximum aggregation rate nor collagen-induced platelet ATP release were significantly different between the groups. According to these results, short-term training caused an alteration in platelet functions limited to the secretion response, which may be related to the oxidant/antioxidant balance changes favoring the antioxidants. The improved plasma total antioxidant capacity was possibly sufficient to prevent exercise-induced oxidative damage, and the adaptive response of platelets might be associated with enhanced antioxidant status.

Key words
Exercise • Antioxidant capacity • Platelet • Aggregation • Secretion

Introduction

A physically active life style has an important role in preventing thrombotic events and decreasing the risk of cardiovascular disease (Berlin and Colditz 1990, Folsom *et al.* 1997). Platelets are involved in the pathogenesis and progression of cardiovascular diseases. One of the beneficial effects of regular exercise is to decrease platelet sensitivity and aggregability (El-Sayed 2002).

On the other hand, physical exercise alters oxidant/antioxidant balance which is known to be important in several physiological and pathophysiological events such as intracellular signalling, aging, atherosclerosis, myocardial infarct and ischemia/ reperfusion injury (Ji 1995, Knight 1995, Sen 1995). Limited studies in which plasma total antioxidant status (TAS) was investigated indicate that platelet function

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changes induced by acute and chronic exercise may be related to alterations in antioxidant capacity (Tozzi-Ciancarelli *et al.* 2002, Ficicilar *et al.* 2003, Di Massimo *et al.* 2004). In addition, short-term training for three or five days were shown to protect myocardium and to increase fatigue resistance of the diaphragm by improving the antioxidant capacity (Vincent *et al.* 2000, Demirel *et al.* 2001). Nevertheless, there is no study demonstrating the effects of short-term training program on plasma TAS and its relation to platelet functions. Platelet reactivity and functions can be evaluated by *in vitro* measuring agonist-induced platelet aggregation and ATP release.

The present study was aimed to test the hypothesis that short-term endurance training would rapidly improve plasma TAS and affect *in vitro* platelet functions. The relationship between antioxidant status and platelet responses was also evaluated.

Methods

Animals

Male Sprague-Dawley rats (14- to 16-week-old) weighing 203.8±28.9 g were used in the study. The animals were housed in pairs per cage in a temperature of 20-22 °C and 12-hour light-dark cycle controlled room. Food and water were provided *ad libitum*. The rats were allowed to rest in their home cages for a week after their arrival. All animal experiments were conducted under the guidelines on humane use and care of laboratory animals for biomedical research published by NIH (No. 85-23, revised 1996). This study protocol was approved by the Ethics Committee, Ankara University, School of Medicine. The rats were randomly assigned to control or exercise groups.

Exercise protocol

Exercise protocols were applied using the motor-driven treadmill system for small animals designed by Department of Electronics Engineering, Ankara University. A day before the exercise protocol, all animals in the exercise group were habituated to the treadmill. The exercise protocol was 25 m/min, 0 % grade, 30 min for three consecutive days (Yamashita *et al.* 2001). At the end of the running period, rats were rapidly anesthetized by pentobarbital sodium, i.p. (Nembutal, 50 mg/kg). After complete anesthesia, the abdominal cavity was opened and blood (~5 ml) was collected from the abdominal aorta. One half of the blood sample (2.5 ml) was drawn into a test tube containing 3.8 % trisodium citrate (in a ratio of 1:9) for the platelet function tests and the remaining 2.5 ml was put into heparinized tubes for the TAS evaluation. The control group animals were kept sedentarily in their cages throughout the study and sampled at similar times as exercised rats. All experiments were carried out between 10:00 and 12:00 h.

Platelet functions

Platelet aggregation was evaluated by impedance technique using a Chrono Log Model 560 WB aggregometer in whole blood (Ingerman-Wojenski *et al.* 1983). A pair of platinum electrodes were placed in 450 µl of blood, diluted with 450 µl of 0.9 % saline solution. The aggregometer was calibrated so that 2 cm on the record paper corresponded to an impedance change of 5 ohms. Collagen (3 µg/ml) and ADP (10 µM) were added to the samples. The increase in impedance due to adhesion and aggregation of platelets to the electrodes was recorded. The maximal aggregation rate (MAR) was taken as the slope of the steepest part of the curve expressed as ohm/min (Fig. 1).

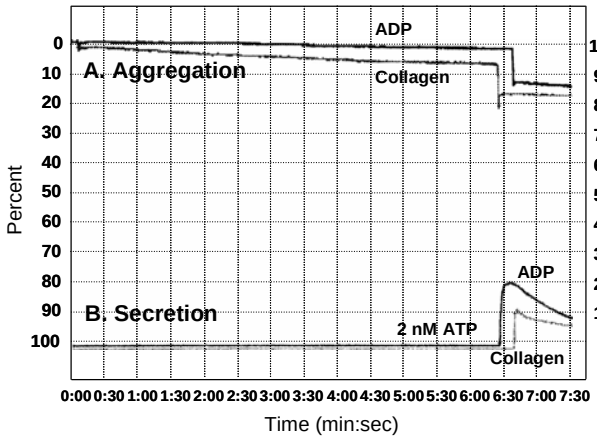

Fig. 1. An example record for the ADP and collagen induced-aggregation and secretion curves. A. Maximum aggregation rate was calculated using tangent of the angle at the steepest part of the slope of aggregation curve and expressed as ohm/min. B. The amount of ATP release was calculated by comparing to the standard curve for 2 nM ATP and expressed in nM.

Platelet ATP release was measured by bioluminescence (Ingerman-Wojenski *et al.* 1983). 100 µl of luciferin-luciferase was added to the samples just after the aggregation had been completed. Luciferin is a phosphored buffer compound, which is emitting light if there is an ATP in the solution. The change in light emission detected at 2 nM ATP of final concentration was used as a standard to quantify ADP or collagen-

induced ATP release of the samples (Fig. 1). ADP, collagen, ATP standard and luciferin-luciferase reagents were obtained from Chronolog Corp (PA, USA). Platelet numbers were counted by using a Coulter Counter T 890 (USA).

Plasma total antioxidant status

After drawing blood into heparinized tubes, plasma samples had been freshly separated and stored at –80 °C until the total antioxidant status measurement was run. Based on the method of Miller *et al.* (1993), the assay was performed by adapting the commercially available kit (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK) to an autoanalyzer (Technicon). In this assay, incubation of ABTS (azino-diethyl-benzthiazoline sulphate) with a peroxidase (metmyoglobin) and H_2O_2 resulted in the production of a radical cation $ABTS^+$, which has a blue-green color that was measured at 600 nm. Antioxidants suppress this reaction, which is proportional to their concentrations in the added sample. The suppression of the color change by plasma samples was compared to the effect of 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid as a standard using graded doses.

Statistical analysis

Platelet functions (ADP and collagen-induced MAR and ATP release) and plasma TAS were analyzed using the non-parametric Mann-Whitney U test. Platelet counts of two groups were compared by using Student's t-test. The correlations between TAS and platelet responses were analyzed with the Pearson correlation test. Differences were considered significant at $p<0.05$ in the bilateral situation.

Results

The mean values of plasma total antioxidant status (TAS), platelet numbers, maximal aggregation rate (MAR), ATP release of the control and short-term training groups are shown at Table 1.

Short-term training decreased ADP-induced platelet release and enhanced plasma TAS ($p<0.001$). No significant differences were found between the groups with regard to ADP- and collagen-induced maximum aggregation rates and collagen-induced platelet ATP release. Significant negative correlation ($r=-0.56$, $p<0.05$) was found between plasma TAS and ADP-induced platelet ATP release. The numbers of platelets among the groups were not statistically different.

Table 1. The mean values of plasma TAS, platelet count, ADP- or collagen-induced platelet maximal aggregation rate and platelet ATP release of the control and short-term training groups.

	Plasma TAS (mmol/l)	**Platelet count ($x10^3/mm^3$)**	**ADP-MAR (Ω/min)**	**ADP-ATP release (nM)**	**Collagen – MAR (Ω/min)**	**Collagen-ATP release (nM)**
Control	1.05±0.1 (n=24)	220±93.8 (n=14)	1.64±1.0 (n=14)	2.18±1.6 (n=14)	1.93±1.6 (n=14)	0.69±0.6 (n=14)
Short-term training	1.96±0.3* (n=21)	172.9±33.9 (n=10)	1.47±1.5 (n=19)	0.26±0.4* (n=19)	2.53±1.4 (n=19)	1.17±0.9 (n=19)

The values are expressed as mean ± S.D. TAS: total antioxidant status, MAR: maximal aggregation rate, ATP: adenosinetriphosphate. * $p<0.001$, compared to the control group.

Discussion

In the present study, we found an increase in plasma total antioxidant capacity and a decrease in platelet secretion induced by ADP in animals subjected to a three-day training program. Both results were statistically significant and a moderate correlation between these two results was detected. There were no significant differences between control and exercise groups considering platelet numbers, maximum aggregation rates and collagen-induced platelet ATP secretion.

Measuring plasma total antioxidant status is a sensitive and reliable marker in evaluation of the effects of different treatments such as exercise on plasma redox status (Ghiselli *et al.* 2000). Plasma distributes a large number of various antioxidants throughout the body which are difficult to be analyzed individually. Further-

more, plasma has a cumulative antioxidant potency due to the interaction of these low molecular weight substances (Prior and Cao 1999). Therefore it is accepted that the methods which measure the cumulative effect of all known and unknown antioxidants present in the plasma provide biologically more relevant information (Prior and Cao 1999, MacKinnon *et al.* 1999, Ghiselli *et al.* 2000).

It is well known that different kinds of exercise generate different levels of oxidative stress (Sen *et al.* 1994, Sen 1995, Liu *et al.* 2000), and that long-term training improves the antioxidant defense (Powers and Lennon 1999, Liu *et al.* 2000, Di Massimo *et al.* 2004).

It was shown that short-term endurance training of 3-5 days caused a protective effect in the myocardium against ischemia/reperfusion damage in rats. This effect was thought to be related to the improvement of cardiac antioxidant defense (Locke *et al.* 1995, Taylor *et al.* 1999, Demirel *et al.* 2001). In addition, Vincent *et al.* (2000) proved that enzymatic and non-enzymatic antioxidants of diaphragm were increased by short-term exercise. It was reported that even a bout of acute exercise which was exerted by Wistar rats at the intensity similar to that of our study played a role in the myocardial protection by Mn-SOD activation (Yamashita *et al.* 1999). Oxidative stress induced by acute or chronic exercise elicits different responses depending on the organ tissue type and its endogenous antioxidant levels (Liu *et al.* 2000). It may be expected that these responses occur in the plasma because of its central role in antioxidant defense. Similarly, Subudhi *et al.* (2001) showed increased plasma total antioxidant capacity following strenuous 2-day exercise in elite skiers. The findings mentioned above consistent with the increase in antioxidant capacity by short-term training demonstrated in the present study.

Only a few studies investigated the relationship between oxidative stress directly related to exercise and platelet activation. In a study with acute intense exercise, oxidatively modified LDL was thought to increase platelet activation which was associated with insufficient total antioxidant capacity (Tozzi-Ciancarelli *et al.* 2002). The previous study performed in our laboratory also revealed that acute intense exercise caused a decrease in plasma total antioxidant status and an increase in collagen-induced platelet secretion response (Ficicilar *et al.* 2003).

The results of exercise studies strictly depend on the type, intensity, duration and frequency of the exercise. Hence, the variations in findings are to be expected with different exercise protocols. In contrast to acute exercise, Wang *et al.* (2000) showed enhanced LDL resistance to oxidation and decreased platelet aggregability in Wistar rats subjected to 10-week chronic exercise program. When healthy and sedentary human subjects were trained at moderate intensity for 20 weeks, their TAS increased and correlated with decreased platelet responses to ADP and collagen (Di Massimo *et al.* 2004).

The effects of short-term training on platelet functions have not yet been investigated together with TAS. In this manner, the present study fills an important gap in literature. Moderate exercise applied for three consecutive days leads to an important increase in TAS and decrease in ADP-induced platelet secretion. These two results were also correlated significantly with each other. On the basis of these findings, we concluded that three-day exercise caused adaptation in platelets limited to the secretion response, which may be related to the oxidant/antioxidant balance changes favoring the antioxidants. If the exercise-induced free radical production does not balance with plasma antioxidants, LDL oxidation occurs. Plasma LDL obtained from trained subjects was found to be more resistant to oxidative modification (Sanchez-Quesada *et al.* 1997). The increased plasma cumulative antioxidant potential observed in our study was possibly enough to scavenge the reactive oxygen radicals produced during exercise. Unfortunately, the oxidative markers were not determined in this study due to technical limitations.

The collagen-induced platelet ATP secretion was not significantly different between the exercised and control groups while there was a difference in ADP-induced platelet ATP secretion. This can be explained by the different platelet responses to ADP and collagen. It is well known that the mediators activating or inhibiting platelet aggregation or secretion use various signaling pathways with different sensitivity (El-Sayed *et al.* 2000, Krötz *et al.* 2002).

In conclusion, the present study has demonstrated an increase in plasma total antioxidant capacity and a relevant decrease in platelet secretion response to *in vitro* agonists in Sprague-Dawley rats subjected to treadmill running protocol with moderate intensity for three consecutive days. According to the results, short-term training caused an adaptation in platelets limited to the secretion response, which may be related to oxidant/antioxidant balance changes favoring the antioxidants. We suggest that the increased total antioxidant capacity may prevent oxidative injury

induced by exercise and alteration in platelet responses to training may be related to improved antioxidant status.

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