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Changes in Blood Haemorheological Parameters After Submaximal Exercise in Trained and Untrained Subjects

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Summary

The blood stream is affected by viscosity and many other haemorheological factors such as lipid peroxidation in the plasma and red blood cells. The aim of this study was to investigate the changes of haemorheological parameters after submaximal exercise in trained and untrained subjects. The results indicated that heart rate, lymphocyte count, erythrocyte deformability, plasma lipid peroxide levels and erythrocyte glutathione peroxidase activity are increased after submaximal exercise.

Key words
Submaximal exercise – Glutathione peroxidase – Lipid peroxidation – Haemorheology

Introduction

Blood is a complex fluid and has some specific dynamics of its circulation. There are many factors affecting the bloodstream such as plasma proteins, red cell number and deformability, leucocyte number, haematocrit etc. Erythrocytes must change their shape in order to pass into the microcirculation. The study of erythrocyte deformability has been attracting growing interest in recent times. Plasma viscosity is another factor which depends on the protein components, particularly fibrinogen, which influence blood viscosity at low shear rates through reinforcement of rouleaux formation (Mokken *et al.* 1992).

According to Shiga *et al.* (1990), a 10 % increase in fibrinogen concentration accelerates the rate of aggregation by 18 %. Whole blood viscosity has been shown to increase with the exercise and it was determined by the erythrocyte deformability, erythrocyte aggregation and haematocrit (Vandewalle *et al.* 1988). As an oxygen carrier, erythrocytes and many other blood cells are apparently affected by oxygen radicals. The addition of malondialdehyde (5–20 μM), a lipid product of oxygen radicals, to human erythrocytes markedly decreases cellular deformability (Pfafferott *et al.* 1982).

On the basis of these results, it was decided to investigate the changes in haemorheological parameters after acute exercise which was assumed to cause radical alterations of blood cells and the plasma.

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Material and Methods

This study was performed on eight male athletes with an average training period of 5 years and age-mean age of 21 years. Blood samples were drawn by venipuncture before and immediately after submaximal bicycle ergometer exercise. The exercise protocol was performed for 30 min with a load of 75 % of individual VO_{2max}. Blood samples served for the determination of the following parameters: the number of red and white blood cells, lymphocytes and platelets, haemoglobin, haematocrit, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean platelet volume which were determined by using a haematologic analyzer (Coulter Model S Plus Vl).

Erythrocyte deformability measurements were performed by using a constant pressure filtration technique. Cells were washed twice in an isotonic Tris-NaCl buffer at pH 7.4. The buffy coat was removed. Erythrocytes were suspended in the buffer at 10 % haematocrit values. The filtration time of 1 ml of erythrocytes through polycarbonate membranes (pore size 4.7 μm Nucleopore-Hemafil, Lot: 54B6A7, 25 mm diameter) under a 5 cm H_2O pressure gradient was determined electronically. The deformability index was calculated by dividing 1 ml cell free buffer through the same filter as follows:

$$\text{Deformabilitiy index} = \frac{\text{Filtration time of 1 ml erythrocyte suspension}}{\text{Filtration time of 1 ml cell-free buffer}}$$

Fibrinogen levels were determined using the coagulometer. Erythrocyte malondialdehyde levels were measured according to Uchiyama and Mihara (1978) and erythrocyte glutathione peroxidase values were determined according to Paglia and Valentine (1967).

Erythrocyte aggregation rates were measured at stasis (m) and at 3 s^{-1} (m_1) in trained and untrained subjects. Fasting venous samples were collected according to standardized protocols (Ernst *et al.* 1985). The measurements were performed in duplicate samples. Erythrocyte aggregation (EA) was studied with a photometric rheoscope (Myrenne aggregometer) which measures EA in 20 μl of blood anticoagulated with EDTA. The estimations were carried out at haematocrit values adjusted to 45 % by removing or adding autologous plasma after shearing at 600 s^{-1} at stasis (m) and at 3 s^{-1} (m_1).

Statistical analysis was performed by analysis of variance and paired Students' t-test.

Table 1. Age and heart rate in sedentary controls and trained athletes in the resting state (R) and after exercise (E)

	Age (years)	Heart rate (beats/min)	
		R	E
Controls	20.1±1.8	80.7±16.0	152.5±9.8*
Trained	22.4±2.3	78.1±13.6	154.9±15.7*

*The values are means ± S.D. * significantly different ($p<0.05$) from the resting state*

Table 2. Erythrocyte malondialdehyde (MDA), glutathione peroxidase (GSHPx) and deformability in controls and trained athletes ar rest (R) and after exercise (E)

	MDA (nmol/g Hb)		GSHP (nmol/dk/g Hb)		Deformability	
	R	E	R	E	R	E
Control	153±26	112±20*	32±4.56	26±2.09*	1.90±0.28	2.35±0.36*
Trained	140±39	103±16*	30±3.47	23±3.78*	1.93±0.30	2.30±0.40*

*The values are means ± S.D. * significantly different ($p<0.05$) from the resting state*

Results

The heart rate at rest was increased in both groups but there was no difference between trained and control subjects after exercise (Table 1).

After exercise, erythrocyte deformability significantly increased, whereas erythrocyte malondialdehyde (MDA) and glutathione peroxidase (Gpx) levels decreased in both groups (Table 2).

During the resting period, mean corpuscular haemoglobin values were different in the trained and untrained groups (Table 3).

White cell and platelet counts were increased after exercise in both groups (Table 4).

Lymphocyte counts had a tendency to higher values in both groups after exercise but this rise was significant in the trained groups only, showing an increased immunological resistance in the trained group (Table 4).

Table 3. Erythrocyte (RBC), haemoglobin (Hb), haematocrit (Htc) mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) in controls and trained athletes at rest (R) and after exercise (E)

	RBCx10^6 mm^3		Hb (g/dl)		Htc (%)		MCV (μ^3)		MCHC (pg)	
	R	E	R	E	R	E	R	E	R	E
Control	5.313	5.400	16.12	16.52	46.78	48.12	88.35	85.32	30.4	30.3
	±0.410	±0.399	±0.77	±0.77	±2.14	±2.12	±5.15	±4.15	±1.74	±2.0
Trained	5.375	5.550	15.63	16.67	46.16	48.04	88.14	88.63	28.9×	30.0
	±0.284	±0.318	±0.71	±0.97	±1.78	±2.28	±5.32	±4.68	±1.05	±1.72

The values are means ± S.D. # significantly different ($p<0.05$) from controls

Table 4. Leukocyte (WBC), platelet and lymphocyte counts in controls and trained athletes at rest and after exercise

	WBC/mm^3		Platelet count x 10^3 mm^3		Lymphocyte (%)		Lymphocyte Count/mm^3	
	R	E	R	E	R	E	R	E
Controls	7 782	9 727	268.1	304.9	33.67	31.54	2.530	2.930
	±1 770	±1 816	±60.9	±63.4	±4.15	±4.82	±700	±700
Trained	7 158	9 200	247.2	298.1	33.72	36.05	2.240	3.210*
	±2 300	±2 422	±36.3	±63.4	±8.68	±6.35	±200	±700

*The values are means ± S.D. * significantly different ($p<0.05$) from the resting state*

Table 5. Aggregation and fibrinogen levels in controls and trained groups at rest and after exercise

	Slow aggregation (m)		Quick aggregation (m_1)		Fibrinogen (mg/dl)	
	R	E	R	E	R	E
Controls	3.623	3.359	5.881	5.847	228	217.3
	±1.210	±1.203	±2.213	±2.277	±45.5	±56.3
Trained	3.158	3.548	7.327	6.887	220.4	254.3
	±1.198	±0.932	±1.935	±1.529	±44.8	±56.0

The values are means ± S.D.

Discussion

The intracellular glutathione (GSH) system in erythrocytes is the most effective antioxidant substance against oxygen-derived free radicals which are a product of intermediate metabolism. Exercise increases cellular metabolism and thus enhances the leakage of oxygen-derived free radicals into the plasma (Duthie *et al.* 1990). Increased red cell filterability and decreased haematocrit values were found in sportsmen compared to the controls. Sedentary subjects have increased red cell filterability after regular exercise (Ernst and Matrai 1987). In our study decreased MDA and glutathione peroxidase levels were observed in the control group immediately after submaximal exercise compared to the resting state.

Glutathione peroxidase activity was first estimated by Mills in erythrocytes (Paglia and Valentine 1967). Ernst *et al.* (1985) showed that blood can be fluidized by regular physical training in sedentary healthy men. They also showed that two months of regular exercise in claudicate persons decreased red cell aggregability and increased the blood cell filterability (Ernst *et al.* 1987). In our study, an increased deformability index was found both in the control and trained group immediately after submaximal exercise.

Controversial results have been reported about plasma malondialdehyde levels. In some studies, increased levels were found but this was not always the case (Alessio 1993). Koz *et al.* (1992) have shown that erythrocyte malondialdehyde levels are increased after acute swimming exercise in rat plasma. In the present study, MDA levels were decreased in erythrocytes after exercise. There is a negative correlation between erythrocyte deformability and lipid peroxidation, such as that caused by free radicals generated in sepsis. This may play a role in the reduction of their deformability. Free oxygen radicals might serve as a possible mediator of the reduction in erythrocyte deformability. antioxidants such as α-tocopherol and fish oil rich in n-3 fatty acids are known to increase the deformability of red cells (Mokken *et al.* 1992).

Duthie *et al.* (1990) found unchanged lipid peroxide values and decreased GSH levels in trained persons immediately after a short run of marathon runners. Their results indicated that alterations in erythrocyte antioxidant status may occur but plasma lipid peroxide levels were not affected. In trained subjects, MCV, MCHC, haemoglobin and blood plasma MDA levels were not changed after half-marathon run (Duthie *et al.* 1990). Exercise training has no effect on the level of free radicals in normal mice, but lipid peroxidation levels increased after exercise in zinc-deficient mice (Cao and Chen 1982). These results confirmed our observations that there was no free radical elevation in erythrocytes of trained subjects.

Ernst *et al.* (1985) proposed that the greater exercise capacity of sportsmen is associated with higher red cell filterability. Red cell aggregation is slightly higher in sportsmen than in control subjects. These results were also confirmed by us because trained subjects had increased aggregation compared to untrained subjects, but this was not statistically significant. Ricci and Masotti (1989) showed that the platelet number is increased and the platelet volume is decreased after exercise. Our results confirmed these findings especially in trained subjects. Vandewalle *et al.* (1988) found that the erythrocyte deformability and erythrocyte aggregation are unchanged but haematocrit values are increased after 1 h of submaximal exercise. Kretzschmar *et al.* (1991) reported unchanged GSH, GSSG and lipid peroxide levels in the plasma of untrained subjects after a bicycle ergometric test as compared to values before the test. Nevertheless, they found decreased GSH and lipid peroxide but unchanged GSSG levels in trained persons. They suggested that GSH levels and lipid peroxidation were affected during exercise irrespective of training.

The number of leukocytes increased in response to stressful stimuli including exercise. Their source is in the marginated pool that is located along vessel walls. In addition, the leukocyte number is affected by demarginated leukocytes from the pulmonary microvascular pool in response to ventilation (Fairbarn *et al.* 1993). Acute bouts of exercise (VO_{2max} >60ml.kg^{-1}.min^{-1}) caused a significant increase in leukocyte number including the lymphocyte count (Hansen *et al.* 1991, Ndon *et al.* 1992). These findings are also in agreement with our results. The heart rate as well as red cell, platelet and lymphocyte counts were increased in both groups after exercise as was expected (Tables 1 and 4).

It is concluded that haematological and haemorheological parameters did not differ in trained and untrained subjects before or immediately after submaximal exercise, but there were some changes associated with exercises in the same group. Erythrocyte GSH-Px and MDA levels decreased while erythrocyte deformability increased after the exercise. These results confirm the beneficial effects of exercise.

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