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Effect of acute exercise on some haematological parameters and neutrophil functions in active and inactive subjects

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Abstract In this work we studied the possible effects of acute exercise on some haematological parameters and on some functions of neutrophils in seven active and six inactive subjects. Physical exercise (10 min on a cycle ergometer at a heart rate of 150 beats · min^{-1}) induced a significant increase in total leucocyte, lymphocyte and neutrophil concentrations in active subjects; serum iron and ferritin concentrations were lower in active compared to inactive subjects. Cellular adhesion, bactericidal activity and superoxide anion production did not change after exercise, while we also observed some differences between active and inactive subjects before exercise. In particular, the neutrophils from active subjects showed a significantly higher percentage of adhesion, higher bactericidal activity and lower superoxide anion production. In conclusion, the training induced changes in some neutrophil functions, while acute exercise influenced, overall, leucocyte concentrations.

Key words Physical exercise · Neutrophils
Serum iron

Introduction

It has generally been accepted that physical exercise induces a series of changes in the immune response (Keast et al. 1988), but it has been shown that the timing and intensity of exercise are important in determining how exercise influences the immune system in healthy subjects (Nehlsen-Cannarella et al. 1991; Nieman et al. 1991). Reports have suggested that, while moderate exercise may enhance resistance to common infections, the effects of excessive physical activity may be detrimental (Hoffman et al. 1962; Mackinnon and Tomasi 1986; Midtvest and Midtvest 1982; Roberts 1986). Acute physical exertion may induce physiological changes in the quality and quantity of circulating white blood cells, accompanied sometimes by a fall in the serum concentrations of iron. Moreover, the effect on the immune response of physical exercise, when it is performed by untrained people, is still not very clear.

Neutrophils represent 50%–60% of the total circulating white blood cells and constitute the first line of defence against foreign organisms.

The present work investigates whether a single episode of acute exercise could alter the capacity of neutrophils to produce the anion superoxide, modifying their adhesion and bactericidal activity. We examined whether the effects of acute exercise are different in active and inactive subjects and whether the altered response may be related to changes in serum iron (SI), ferritin (FERR) and transferrin (TRF) concentrations.

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Methods

Subjects

Six inactive and seven active healthy subjects, aged from 21 to 25 years, participated in the study after being informed that it could cause stress. All inactive subjects devoted less than 3 h · week^{-1} to exercise-related activities and were nonsmokers and teetotal. The active subjects engaged in regular exercise, consisting of running, swimming and cycling 4–5 days · week^{-1} and were members of the Research Center of physical Fitness and Sports (Verona). The hours taken up in exercise were different for each active subject, ranging between 2 to 4 h · day^{-1}. None had any symptom of illness or infection or had taken drugs in the previous 2 months.

Protocol

On the day of the test, a standardized exercise schedule was undertaken by each participant. The study was performed at 8 a.m. in the fasting state. Peripheral venous blood samples were drawn by antecubital venipuncture immediately before exercise. Each subject exercised on a racing cycle ergometer performing stepwise increases in intensity until a heart rate of 150 beats·min^{-1} was reached within 5 min. This exercise intensity was then maintained for 10 min after which 40 ml of blood was collected. Systolic blood pressure and heart rate were recorded immediately before and after exercise.

Analytical methods

The determination of red blood cell count was made with a Technicon H6000 automatic analyser; SI and TRF concentrations were determined using the Technicon RA 1000 system methods (Hoffman-La Roche, Basel, Switzerland); FERR concentrations was estimated by a fluorometric enzyme immuno-assay (STRATUS, Baxter Co). Total leucocytes and differential counts were estimated by standard procedures (Coulter Counter T660).

Separation of neutrophils

The neutrophils were prepared from ethylene-ethylene diamine tetra-acetic acid anticoagulated blood, that was fractionated by different centrifugations over Percoll gradients. The cells (> 98% of purified neutrophils) were finally resuspended in Hank's balanced salt solution (HBSS) supplemented with 0.2% human serum albumin, 5 mmol·l^{-1} glucose, 0.5 mmol·l^{-1} $CaCl_2$ and 1 mmol·l^{-1} $MgSO_4$ (HCGMA) and used at the concentration of 4×10^6 neutrophils·ml^{-1} for superoxide anion assay and 5×10^6 cells·ml^{-1} for bactericidal activity and maintained at room temperature until use.

Bactericidal assay

A wild strain of Staphilococcus aureus, grown in brain heart infusion for 16 h at 37°C, was used in the experiment. The suspension of bacteria was then centrifuged at 3000 rpm for 10 min and, discarding the supernatant, resuspended in HCGMA. The concentration of bacteria in the suspension was adjusted to obtain an optical density (OD) against a buffer blank at 620 nm of approximately 0.200, corresponding to about 5.12×10^8 bacteria·ml^{-1}. Scalar dilutions 1:1 from the starting suspension (D_0) were prepared until D_7. A 25-µl sample of these dilutions was delivered in triplicate into the wells of a 96-well sterile microplate with a flat bottom (Limbro-type, Flow). Then the wells were supplemented with 25 µl of 16% pooled human sera (diluted in HBSS) and, after 10 min, with 50 µl of the neutrophil suspensions or 50 µl of HCGMA in control samples. The incubation was carried out for 60 min at 37°C in a humidified thermostat, then 25 µl of 0.8% saponin and 100 µl of brain heart infusion were added to all wells including blanks. Bacterial growth was followed at timed intervals (every hour from 0 to 5 h) by reading the OD at 620 nm with a microplate reader. Bactericidal capacity of the neutrophils was evaluated by comparing the bacterial growth of samples incubated with neutrophils to the bacterial growth in the absence of cells.

Superoxide anion assay

The superoxide anion was measured by the superoxide dismutase-inhibitable reduction of ferricytochrome-c modified for microplate assay (Bellavite et al. 1992). As preliminary experiments showed that neutrophils spontaneously adhered to the bottom of the wells and produced considerable amounts of O_2^-, nonspecific activation was totally eradicated by coating the microplate wells with 100 µl of foetal bovine serum for at least 2 h at room temperature: immediately before use, the plates were washed three times with phosphate saline buffer (PBS) using an automatic plate washer (SLT Laboratory Instruments). Operatively, the wells were supplemented with 25 µl *N*-formylmethionylleucylphenylalanine (FMLP) 10^{-7} mol·l^{-1} as a stimulant, 25 µl of cytochrome-c, as the probe for the detection of O_2^-, and finally 50 µl of the neutrophil suspension prewarmed at 37°C for 10 min. As controls, in the wells were added 25 µl of HCGMA, 25 µl of cytochrome-c and 50 µl of the cells without the stimulant. The plates were then brought to 37°C in a humidified incubator throughout the experiment. When indicated (at 5, 10, 20, 30 and 40 min) the plates were rapidly transferred into a microplate reader (READER 400, SLT Laboratory Instruments) and the reduction of cytochrome-c was measured at 550 nm using 540 nm as a reference wavelength to avoid interference due to light scattering. In all procedures, care was taken to avoid cooling of the plate when it was taken from the incubator for readings. To obtain the amount (nanomoles) of superoxide anion produced, the OD of the sample was divided by the OD of the ferricytochrome-c reduced (1 nmol = 0.04 OD).

Adhesion

The test was made immediately after the detection of the superoxide anion. The microplate was subjected to two washing cycles with PBS at room temperature and the cellular adhesion was evaluated by measuring the membrane enzyme acid phosphatase. A quantity of 75 µl of an acetate buffer 0.15 mol·l^{-1}, pH 5.3, containing 0.2% Triton X-100 were plated: after 5 min at room temperature 75 µl of 0.15 mol·l^{-1} acetate buffer containing the substrate (10 mmol·l^{-1} p-nitrophenylphosphate) was added. After incubation at room temperature for 20 min, the reaction was stopped by adding 100 µl of NaOH 2 N. The p-nitrophenol production was measured spectrophotometrically at 405 nm. The percentage of cellular adhesion was calculated on a standard curve obtained with known numbers of neutrophils.

Statistical analysis

The Student's *t*-test was used for comparison between groups.

Results

The haematological parameters in our 13 subjects studied before and after an acute exercise test are shown in Table 1. The total number of leucocytes, lymphocytes and neutrophils significantly increased in the active subjects after exercise; in the inactive subjects the exercise increased all leucocytes, but the difference was significant for lymphocytes only. Eosinophil concentrations were greater in active subjects compared to inactive both before and after exercise. In the active subjects SI and FERR concentrations were lower compared to the inactive ones, even if not significant, before and after exercise: the high inter-subject variability was due to different values between the men and women, with some female subjects showing very low concentrations of FERR.

Table 1 Haematological parameters in our subjects before (B) and after (A) exercise

Parameters	Inactive				Active			
	B		A		B		A	
Red blood cell count	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
$(10^6 \cdot \mu l^{-1})$	5.94	0.26	5.93	0.25	5.12	0.17	5.34	0.21
Serum iron $(\mu g \cdot dl^{-1})$	100.10	11.67	109.20	13.49	78.57	5.29	82.71	5.30
Transferrin $(mg \cdot dl^{-1})$	328.20	26.82	346.31	31.57	346.29	18.39	365.29	18.60
Ferritin $(ng \cdot ml^{-1})$	56.47	17.72	65.90	24.09	44.41	18.60	46.48	18.89
Total leucocytes $(10^3 \cdot \mu l^{-1})$	5.49	0.55	7.53	1.09	6.58	0.54	9.54	0.97*
Lymphocytes $(10^3 \cdot \mu l^{-1})$	1.91	0.15	2.94	0.40*	2.30	0.31	3.20	0.23*
Neutrophils $(10^3 \cdot \mu l^{-1})$	3.13	0.40	3.88	0.63	3.41	0.27	5.12	0.74*
Eosinophils $(10^3 \cdot \mu l^{-1})$	0.07	0.01	0.10	0.02	0.29	0.06**	0.37	0.06**

*$P > 0.05$ Before and after exercise.
**$P < 0.01$ between trained and untrained subjects

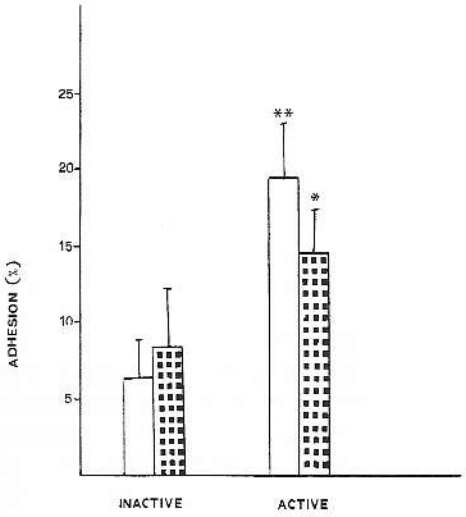

Fig. 1 The percentage of cellular adhesion with *N*-formylmethionyl-leucylphenylalanine 10^{-7} mol·l^{-1} as stimulant before (□) and after (■) exercise in our subjects. The values are expressed as mean and SEM

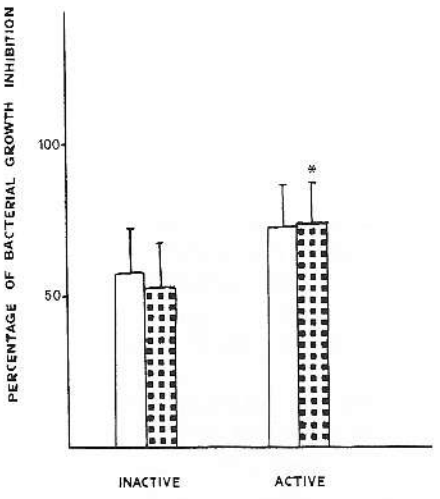

Fig. 2 Bactericidal activity, detected at 5 h, of neutrophils obtained from our subjects before (□) and after (■) exercise. The values are expressed as mean and SD

The percentage of cellular adhesion before and after exercise in our subjects is shown in Fig. 1. We observed a significantly higher percentage of adhesion in the active compared to the inactive subjects whether before or after the exercise, while this parameter of leucocyte function was not influenced by exercise in either group.

The results of the bactericidal assay are shown in Fig. 2. We evaluated the bacterial dilution D_3 since the ratio cells : bacteria produced the best results in this and in previous experiments. The acute exercise test induced significant changes in the percentage of killing bacteria in the active compared to the inactive subjects; in every case the neutrophils from the sedentary subjects showed a lower bacterial activity at rest compared to the active subjects.

The superoxide anion released by neutrophils, with and without the stimulant FMLP, isolated before and after exercise is given in Fig. 3: the values were significantly lower in the active compared to the inactive subjects and the exercise did not significantly influence the superoxide anion release in either group. Compared to values obtained without stimulant, the superoxide anion release in the active and the inactive subjects was significantly higher in the presence of FMLP.

Discussion

It has recently been reported that rigorous training produced a state of immunodeficiency in athletes, causing increased susceptibility to infections (Fitzgerald 1988). However, the precise basis of this phenomenon is not clear. The effect of physical exercise on immune cell function has hardly been investigated, even though it has been widely accepted that quantitative and qualitative alterations of immune cells are produced after physical exercise.

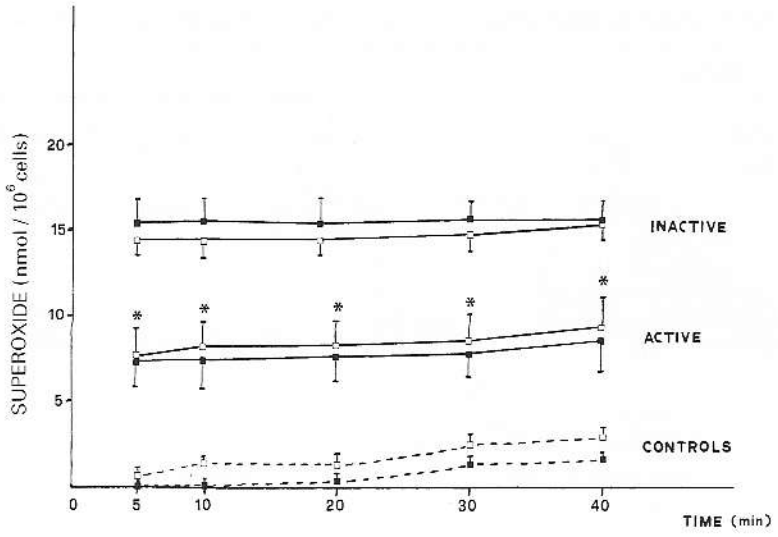

Fig. 3 Superoxide anion production without and with *N*-formylmethionylleucyl-phenylalanine 10^{-7} as stimulant before (□) and after (■) exercise in our subjects. The values are expressed as mean and SEM

In the present work we have studied the effect that acute exercise on a cycle ergometer has on the concentration of circulating blood cells and on some functions of neutrophils in active and inactive subjects: overall we found some differences in neutrophil activity induced by training which were more than those found in the acute exercise.

The SI, FERR and TRF concentrations do not seem to have had any influence, at least in these subjects, on the neutrophil functions. With respect to the number of immune cells, our results showed a significant increase in total leucocytes, in lymphocytes and neutrophils after exercise in the active subjects. These results are in agreement with other published data that have indicated the appearance of leucocytosis after physical exercise (Christensen and Hill 1987; McCarthy and Dale 1988; Oshida et al. 1988). Moreover, the vigorous daily training probably induced the increase in eosinophil concentrations observed, even at rest, in our active subjects: the values were, however, within the normal range and not due to any pathological inflammatory damage as found by Hanson and Flaherty (1981). In inactive individuals, the only significant change in total leucocytes has been found to be the increase in lymphocyte count after exercise, according to other workers (Ortega et al. 1993). The major source of polymorphonuclear leucocytes observed after acute exercise in active subjects has been ascribed to the mobilization of white blood cells from the marginal pool due to haemodynamic redistribution (Athens et al. 1961; Muir et al. 1984) and augmentation by cortisol and catecholamine secretion (Nieman et al. 1991).

In recent years, some authors have found that phagocytic function is stimulated after acute exhausting exercise both in peritoneal macrophages (De La Fuente et al. 1990; Ortega et al. 1992) and in blood neutrophils (Rodriguez et al. 1991).

Our results, firstly, showed that, irrespective of training, the physical exercise enhanced the capacity of circulating neutrophils to produce reactive oxygen species upon stimulation. We observed, moreover, that the training depressed the neutrophil anion superoxide production, compared to sedentary subjects, irrespective of the effects of exercise. This is in agreement with other workers, who have shown that this activity of cells isolated from trained individuals is depressed (Smith et al. 1990), while moderate exercise has also been seen to enhance re-active oxygen species in neutrophils from untrained subjects (Smith et al. 1990).

A correlation between the intensity of re-active oxygen formation and killing capacity may be expected, since patients with severe impairments in this pathway have been shown to suffer from recurrent infections (Curnette et al. 1988).

At rest, it has been shown that there is higher neutrophil bactericidal activity in active than in inactive subjects and this difference becomes significant following acute exercise. Since it is not possible to explain this observation on the basis of a high anion superoxide production, we hypothesized that other nonoxydative mechanisms, which have been found to include mediators such as adrenalin (Bevilacqua et al. 1988), neuropeptides like substance P (Serra et al. 1988) as well as various eicosanoids and lastly nitric oxide (Moncada et al. 1991), could influence this activity.

In our research, a correlation was found to exist between neutrophil adherence and bactericidal activity in active subjects. No significant changes were found in adherence after acute exercise in either group as has been seen by other authors (Lewicki et al. 1987; Ortega

et al. 1993) even when it was found to be higher in active compared to inactive subjects before the exercise.

Other authors have observed a low adherence of neutrophils in trained cyclists (Lewicki et al. 1987) and football players (Seneczko 1983). Probably these dissimilar results are a consequence of the different methods used, of the characteristics of the exercise (duration and intensity) and of the type of athletes tested. Another explanation may be that the adhesion receptors of neutrophils may not be affected by acute exercise performed by untrained subjects as has been described by other authors (Ortega et al. 1993).

The number and/or affinity of neutrophil membrane receptors may be modified by factors such as TNF, for example, which has been found to increase the C_3b receptor expression on neutrophil (Berger et al. 1988), and leucocyte cytotoxic activity has been found to increase in parallel with enhanced C_3b receptor expression after an exercise-induced asthma attack (Moqbel et al. 1986).

The most obvious conclusion to be drawn from this research is that training, more than acute exercise, induces changes in some neutrophil functions and that these are more likely to have a beneficial than a deleterious effect on the phagocytic system. Because neutrophils are involved in both immunity and inflammation, changes in their activity may provide a sensitive marker for overtraining.

Certainly further studied are needed on the response of natural killer cells to various forms of exercise, especially to determine possible mechanisms by which exercise may influence the immunosurveillance and other functions of natural immunity.

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