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Leukocyte, lymphocyte and platelet response to dynamic exercise*

Duration or intensity effect?

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Summary. The influence of work intensity and duration on the white blood cell (WBC), lymphocyte (L) and platelet (P) count response to exercise was studied in 16 trained subjects (22 ± 5.4 years, $\bar{x} \pm$ SD). They performed three cyclo-ergospirometric protocols: A) 10 min at 150 W followed by a progressive test (30 W/3 min) till exhaustion; B) constant maximal work ($\dot{V}_{O_{2max}}$); C) a 45 min Square-Wave Endurance Exercise Test (SWEET), ($n=5$). Arterial blood samples were taken: at rest, submaximal and maximal exercise in A; maximal exercise in B; 15th, 30th and 45th min in the SWEET. Lactate, $[H^+]$, $PaCO_2$, PaO_2, [Hct], Hb, cortisol, ACTH, total platelet volume (TPV), total blood red cell (RBC), WBC, L and P were measured. At 150 W, WBC, L, P, and TPV increased. $\dot{V}_{O_{2max}}$ did not differ between A and B, but a difference was found in total exercise time ($A = 25 \pm 3$ min; $B = 7 \pm 2$ min, $p < 0.001$). In A, at $\dot{V}_{O_{2max}}$, the increase was very small for Hct, [Hb], and RBC (10%), in contrast with large changes for WBC (+93%), L (+137%), P (+32%), TPV (+35%), $[H^+]$ (+39%), lactate (+715%), and ACTH (+95%). At $\dot{V}_{O_{2max}}$ there were no differences in these variables between A and B. During the SWEET: WBC, L, P, TPV and ACTH increased at the 15th min as much as in $\dot{V}_{O_{2max}}$, but no difference was observed between the 15th, 30th and 45th min, except for ACTH which continued to rise; the lactate increase during the SWEET was about half (+341%) the value observed at $\dot{V}_{O_{2max}}$, and $[H^+]$ did not vary with respect to values at rest. The data show that the increase in WBC, L, P, and TPV is small in submaximal exercise but larger at $\dot{V}_{O_{2max}}$ and during the SWEET; high intensity plays a greater role than the total time of exercise in the variation, and haemoconcentration, cortisol and acidosis seem to have little influence.

Key words: White blood cells — Lymphocytes — Platelets — Hemoglobin — Lactate — Cortisol — ACTH — Acid-base balance — Exercise testing — Normal trained men

Introduction

In normal men muscular work of varying duration and intensity produces a leucocytosis (Anderson 1955; Ahlborg 1967), the total white blood cell count (WBC) being higher than 10000 mm^{-3}. A similar observation has been made during endurance running (Dickson et al. 1982; Vishnu Moorthy and Zimmerman 1978). The lymphocyte response to exercise tests and other sports activities is controversial (Anderson 1955; Ahlborg 1967; Vishnu Moorthy and Zimmerman 1978).

The platelet count may increase significantly following strenuous activity (Ohri et al. 1983) but may not change after mild prolonged activity (Bennet 1972; Pegrum et al. 1976). Information about the response of white blood cell (WBC), lymphocyte (L) and platelet (P) counts to submaximal and maximal exercise in the same subject are scarce, and it is not clear how such responses are related to the type, duration and/or intensity of exercise.

The aim of this work was to study the leukocyte, lymphocyte and platelet count responses to submaximal and maximal exercise and to work duration in normal trained subjects.

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Subjects and methods

Sixteen trained normal subjects volunteered for this study after having been informed of the protocol involved. They had practiced sport for about 6 hours per week for more than three months. They completed a medical questionnaire and underwent a cardiopulmonary and electrocardiographic examination, all of which were normal for all subjects. None were smokers and no subject was overweight. Maximal oxygen uptake ($\dot{V}_{O_{2max}}$) was measured in duplicate, as described previously (Gimenez et al. 1981).

The subjects arrived at the laboratory at 8 a.m., after taking a light breakfast with no fat content 2 h before. After half an hour of rest in a lounge chair, a soft catheter was placed in the radial artery with or without local anesthesia. The first blood sample was taken after 15 to 20 min of rest. The subject was then seated on a bicycle ergometer (Jaeger) and carried out one of the series of exercise tests: the subject performed the rest of the exercise tests on alternate days.

The exercise tests

1. The trapezoidal test began at 150 watts for 10 min, and this was followed without interruption by a progressive test of 30 W/3 min. The maximal tolerated power over three minutes (MTP) was taken to be maximal oxygen uptake ($\dot{V}_{O_{2max}}$) if the subject stopped pedalling at the end of that particular step. However, if the subject carried on to a higher load even though the he was unable to maintain the effort for 3 min (MTP + 30 watts), this maximal level was considered as $\dot{V}_{O_{2max}}$ (Gimenez et al. 1981).

2. A maximal constant test at MTP for as long as the subject could tolerate. Subjects usually had to give up this exhausting test after 5 to 10 min, which still permitted us to obtain $\dot{V}_{O_{2max}}$ (Gimenez et al. 1981). The respiratory variables during the last minute of exercise were used. The blood samples were taken at rest and at the end of exercise.

3. The Square-Wave Exercise Endurance Test (SWEET), of 45 min duration, consisted of a submaximal base level, chosen as described by Gimenez et al. (1982), on which a 60 s peak at MTP was superimposed every 5 min. The peak was achieved by increasing both the load and the pedalling rate from 60 rpm to 90 rpm, to simulate a true acceleration. The submaximal base level corresponds to the largest percentage of MTP which could be maintained for 45 min. It is referred to below as the maximum intensity of endurance or MIE_{45} (Gimenez et al. 1982). Blood samples were taken at the end of the 15th, 30th and 45th minutes of the SWEET. This test was performed in 5 subjects only.

Ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}_{O_2}$), CO_2 output ($\dot{V}_{CO_2}$) and respiratory rate were measured continuously using a Jaeger Ergopneumotest with a Dataspir EDV 70 data processing system (E. Jaeger, Würzburg, FRG). The electrocardiogram was continuously monitored on a screen and recorded (Hellige-France, Multiscriptor EK 33).

The following measurements were made on the blood samples: blood gases, using an ABL Radiometer (pH, PaO_2, $PaCO_2$); lactic acid by an enzymatic technique (Hartley et al. 1972); total white blood cell (WBC) count, mean corpuscular volume (MCV), platelet count (P), lymphocyte count (L), total platelet volume (TPV), corrected haematocrit (Hct) and whole blood haemoglobin concentration [Hb], in an electronic Coulter Counter S-Plus; ACTH and cortisol were measured by a radioimmunoassay technique (Hasler et al. 1976; Kchlet and Binder 1973). All hormonal assay were carried out in duplicate. Quality controls were included in all sets of measurements.

The paired *t*-test was used to correlate the differences between values at rest, submaximal and maximal levels of the trapezoidal test, and between rest and the 15th, 30th, and 45th min of the SWEET. An analysis of variance was also used to test time variations during the SWEET.

Results

The physical characteristics ($\bar{x} \pm$ S.D.) of the group were: age, 22 ± 5.4 years; height, 177 ± 5.3 cm and weight 68.5 ± 9.5 kg.

Trapezoidal test

At the maximal level of exercise (308 ± 30 W), heart rate corresponded with predicted values (220-age) and the respiratory exchange ratio ($\dot{V}_{CO_2}/\dot{V}_{O_2}$) exceeded 1.10 in all subjects. Table 1 shows the mean values of arterial blood gases, acid-base balance and haematological variables at rest, 150 watts and $\dot{V}_{O_{2max}}$. Considering the differences between rest and submaximal exercise, $[H^+]$, $PaCO_2$, PaO_2, [Hb], Hct, RBC, cortisol and ACTH did not change, while an increase was observed in WBC (+32%), L (+37%), P (+10%) and TPV (+11%) (Fig. 1). With respect to values at rest, there was, at maximal $\dot{V}_{O_2}$ (65 ± 8

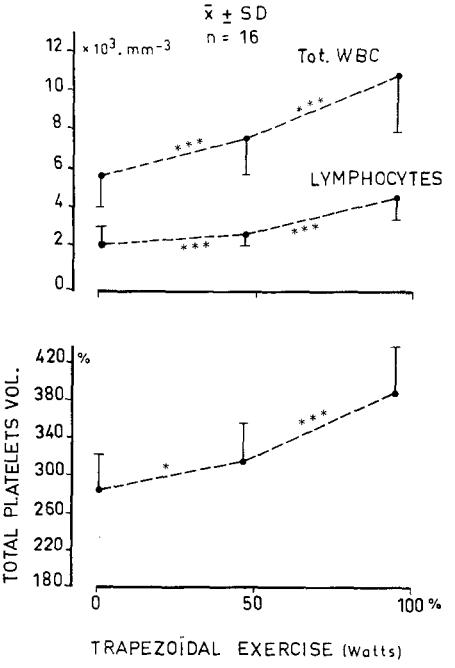

Fig. 1. Changes in lymphocytes, white blood cells and total platelet volume at rest, at 50 and 100% of maximal exercise during the trapezoidal test. * $p < 0.05$; *** $p < 0.001$

Table 1. Arterial blood gases, acid-base balance and haematological variables at rest and exercise in 16 trained subjects

	Rest	Exercise				
		Trapezoidal			Constant	
		150 W (10 min)		$\dot{V}_{O_2max}$		$\dot{V}_{O_2max}$
$[H^+]$ nmol · l^{-1}	37 ± 3.2	40.2 ± 1.7	***	51.3 ± 5.5	49	± 5.2
$PaCO_2$ mmHg	37.5 ± 3.5	40.5 ± 1.9	***	32.9 ± 2.9	32	± 2.3
PaO_2 mmHg	98 ± 8	90 ± 5		81 ± 6	82	± 6.6
[Hb] g · dl^{-1}	14.9 ± 0.9	15.4 ± 1.1	**	16 ± 0.9	16.1	± 0.9
Hct %	44.5 ± 2.6	45.9 ± 3		48.4 ± 2.4	48.2	± 2.2
RBC ($\times 10^6$ · mm^{-3})	5.0 ± 0.4	5.2 ± 0.4	*	5.4 ± 0.4	5.38	± 0.4
Platelet ($\times 10^3$ · mm^{-3})	265 ±38	* 291 ±39	**	350 ±48	342	±53
Cortisol ng · dl^{-1}	23 ± 9.6	21.6 ± 8.5		20.6 ± 8.8	27.8	± 6.8
ACTH pg · ml^{-1}	46.5 ±63	42.9 ±51	***	90.7 ±65	71	±51
Lactate mmol · l^{-1}	1.24± 0.56	1.51± 1.12	***	10.1 ± 2.6	11.2	± 2.1

* $p<0.05$; ** $p<0.01$; *** $p<0.001$; $[H^+]$ arterial H^+ concentration; $PaCO_2 - PaO_2$ = carbon dioxide and oxygen arterial pressure; [Hb] = haemoglobin concentration; Hct = haematocrit; RBC = red blood cell
Values are mean ± SD

ml · kg^{-1} · min^{-1}) a marked acidosis, ($[H^+]$ = +39%; lactate = +715%) with a decrease in $PaCO_2$ (Table 1). The increase in [Hb] (+7.4%), Hct (+8.8%) and RBC (+8%) was very small, in contrast with large changes in WBC (+93%), L (+137%), P (+32%), TPV (+35%) and ACTH (+95%). Cortisol did not change at $\dot{V}_{O_2max}$ (Table 1).

Maximal constant test

The $\dot{V}_{O_2max}$ observed with this test was practically the same as that of the trapezoidal test (63.6 ± 6

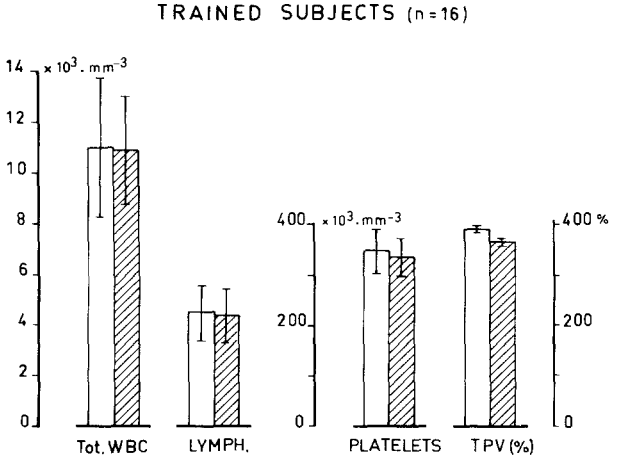

Fig. 2. White blood cells (WBC), lymphocytes, platelets and total platelet volume (TPV) during two maximal exercises of different durations ($p<0.001$). No difference is seen in the hematological variables considered. □ Maximal work. Trapezoidal (25±3 min); ▨ Maximal work. Constant (7±2 min) $\bar{x}$ ± SD

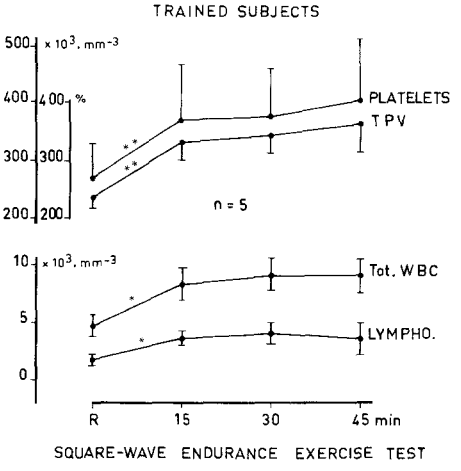

Fig. 3. Lymphocytes, white blood cells, total platelet volume and platelets during the 45 min Square-Wave Endurance Exercise Test

ml · kg^{-1} · min^{-1}). Also, no difference was found between $[H^+]$, $PaCO_2$, PaO_2, [Hb], Hct, WBC, L, P, TPV, ACTH and cortisol (Fig. 2, and Table 1) during the trapezoidal and the constant maximal exercises. However, the total exercise time was significantly different ($p<0.001$) between the trapezoidal (25±3 min) and the constant (7±2 min) tests.

Square-wave endurance exercise test

The mean values of P, TPV, WBC and L are presented in Fig. 3. All of these variables, and [Hb], Hct, RBC, rose significantly from rest to the 15th min of exercise. However no difference was ob-

served between the 15th, 30th and 45th min either by the paired *t*-test or by analysis of variance. Cortisol did not change at any time during the SWEET (rest = 19.4 ± 10.3; 15th min = 17.2 ± 8; 30th = 20.2 ± 6.3; 45th min = 24.5 ± 11 $\mu g \cdot dl^{-1}$) while ACTH increased progressively (rest = 54 ± 42; 15th min = 75 ± 52; 30th min = 135 ± 112; 45th min = 158 ± 105 $\mu g \cdot ml^{-1}$). Lactate (rest = 1.73 ± 0.4 $mmol \cdot l^{-1}$) rose at the 15th min (5.47 ± 1.5 $mmol^{-1}$) and thereafter did not change significantly (30th min = 5.38 ± 1.27; 45th min = 6.34 ± 1.35 $mmol^{-1}$). The arterial $[H^+]$ did not vary throughout the SWEET and was in the normal range (37—43 $nmol \cdot l^{-1}$) (Gimenez et al. 1982).

Discussion

The main features of these results are the marked increase in WBC, L, P, TPV and ACTH observed during the different maximal exercise tests, despite the differences in time required.

Our findings on WBC during the trapezoidal and constant maximal tests are in agreement with previous series (Anderson 1955; Ahlborg 1967; Vishnu Moorthy and Zimmerman 1978; Dickson et al. 1982). Anderson (1955) demonstrated, after three minutes intensive exercise on a treadmill, a 40 to 120% increase in WBC. Other studies dealing with long distance running (Oyster 1980; Dickson et al. 1982; Wells et al. 1982) also showed a marked increase in WBC. Our study shows that leukocytes increase at submaximal levels, with a marked increase at $\dot{V}_{O_2max}$. It seems, therefore, that the maximal increase in WBC is more related to the maximal intensity of exercise than to its duration (Fig. 2 and 3). However, Ahlborg (1967) found a relationship between leukocytosis and exercise duration: at a level representing about 50% of maximal exercise, he observed an increase of 88% at the 10th min. This is disproportionately more than in other studies (Duner and Pernow 1958; Shoenfeld et al. 1981) and in ours (Fig. 1). The absolute WBC value found by Ahlborg at the end (180 min) of this moderate exercise was extremely high ($> 25\,000\ mm^{-3}$). Substantially lower values were observed by others, even after exhaustive long distance races (Vishnu Moorthy and Zimmerman 1978; Dickson et al. 1982). The differences could be related in part to the blood sample collecting technique and also to the method of counting. At the maximal level of trapezoidal and constant exercises (Fig. 2) the WBC counts are very similar, while the total exercise times are significantly different. In addition, during the SWEET, at MIE_{45} no significant variation in WBC was detected by the variance test between the 15th, 30th and 45th minutes (Fig. 3). It seems therefore that, at least with the different maximal protocols used in this study in trained subjects, time does not play a role in the leukocytosis of maximal exercise.

The relationship between lymphocytes, platelets and the duration or intensity of exercise is more controversial. Steel et al. (1974) showed that physical exercise undertaken in normal non-manual conditions contributes little to physiological fluctuations in peripheral blood lymphocytes in health. The increase in lymphocytes with exercise observed in the literature varied, being 8% (Duner and Pernow 1958), 31% (Shoenfeld et al. 1981), 38% (Vishnu Moorthy and Zimmerman 1978) 88 and 148% (Ahlborg 1967). This large variability in response seems to be related to the type of exercise. In this study the absolute values, for lymphocytes are very similar in the three protocols despite differences in their duration. Moreover, after a 20 mile race, by regular runners, the duration of which varied from 118 to 176 min, Vishnu Moorthy and Zimmerman (1978) observed an average increase in lymphocytes of only 38% and a decrease in two out of seven subjects. Wells et al. (1982) in well trained male and female runners, found no change in lymphocytes after a marathon race, with an average time of 199 min for males and 178 min for females. In the light of these results, the duration of exercise does not seem to be an important constant factor in the lymphocytosis of exercise.

A critical amount of exercise is required to produce a detectable rise in P (Dawson and Ogston 1969). With a moderate workload, healthy women had significantly elevated platelet counts immediately after exercise, while no such changes were found in healthy men at the same level of work (Dawson and Ogston 1969). However P rises in men with a higher workload (Dawson and Ogston 1969; Ohri et al. 1983). Significantly elevated platelet counts following strenuous activity were observed by Bennet (1972) and Sarajas et al. (1961) but no variation was found after mild or prolonged activity (Bennet 1972). Our data are in agreement with these results. At maximal $\dot{V}_{O_2}$ our study revealed an increase in P by 32%, which is somewhat higher than the 17 to 25% found by others (Ferguson and Guest 1974; Warlow and Ogston 1974; Ohri et al. 1983) during submaximal exercises, but somewhat lower than the 43% found by Hawkey et al. (1975) during maximal ex-

ercise. TPV also increased significantly in this study.

The origin of the increase in circulating leukocytes, lymphocytes and platelets with exercise is obscure. It has been postulated that the lungs play an important role in the removal of those cells (Sarajas et al. 1976).

A "margination", that is a tendency for leucocytes to congregate along blood vessel walls outside the axial stream of blood flow, has been described by Robbins and Angeli (1976). Any accelerated circulatory activity from exercise may cause their release (Sarajas et al. 1976; Wilkerson et al. 1976). The increased number of these cells is too large to be attributed to haemoconcentration (Ohri et al. 1983; Wells et al. 1982). As it has been demonstrated that the rise in P is also seen in splenectomised individuals (Dawson and Ogston 1969) the spleen is not the only source of this increase. The repeated impact of the feet against the ground in runners might result in an inflammatory response to local tissue injury (Wells et al. 1982). This is not the case during exercises performed on a bicycle ergometer, as in this study.

Acidosis had been quoted as a cause of mobilisation of lymphocytes and platelets (Menkin 1934; Crowell et al. 1961), but the role of this mechanism has not been investigated during exercise. In this study, there was an important lactic acidosis at $\dot{V}_{O_2max}$ (Table 1) while during the SWEET lactic acidosis was moderate and compensated. Consequently lactic acidosis is unlikely to explain all the changes observed.

Another factor to be considered is the response of cortisol. Pre-exercise blood samples might not be representative of a true prolonged rest. Indeed, our cortisol rest values were more elevated than some values given in the literature (Oyster 1980; Kuoppasalmi et al. 1980), but not so different from other values (Maron et al. 1977; Kinderman et al. 1982; Lavoie et al. 1985). Many factors such as anxiety after the arterial catheter was inserted (Maron et al. 1977) and because of the cyclic variations of cortisol, as the time in which the blood sample was taken (Persky 1953), may explain this difference. The evolution of cortisol during exercise is controversial. In some papers, it is shown as rising during exercise (Hartley et al. 1972; Maron et al. 1977; Kuoppasalmi et al. 1980; Vishnu Moorthy and Zimmerman 1978), while in others no change is observed (Oyster 1980; Lavoie et al. 1985). Moreover, after exogenous stimulation of the suprarenal cortex, the plasma cortisol response reaches a maximum 30 to 60 min after initial stimulation, and stays there for about one hour (Kchlet and Binder 1973). This indicates that large increases in ACTH could be found without variation in cortisol levels over the total time of the tests used in this study. Persky (1953) found that ACTH was related to WBC increase. We found that the increase in ACTH at $\dot{V}_{O_2max}$ and during MIE_{45} coincides with that of WBC, L and P. It is likely that the increase in ACTH at maximal exercise, which indicates stress, plays a certain role in these haematological changes.

The present study indicates that in individuals engaged in endurance training, moderate or maximal exercise produces an increase in white blood cells, lymphocytes, platelets and total platelet volume, which seem more closely related to the intensity than to the duration of the exercise. While part of the changes could be due to lactic acidosis, it is also possible that they are related to exercise stress, as manifested by increased ACTH.

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