

EFFECT OF EXERCISE-INDUCED PHYSIOLOGICAL STRAIN ON BLOOD CORTISOL LEVEL AND PERIPHERAL BLOOD LEUKOCYTES IN TRAINED AND UNTRAINED MEN

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- physiological strain,
- blood leukocytes,
- cortisol.

Abstract:

The main purpose of the present study was to examine the effect of single exercise test performed to exhaustion on blood cortisol level and total number and relative proportions of blood leukocytes in untrained and trained men. Thirty-one healthy men were recruited to participate in this study. Nineteen of them were the CrossFit trained (5 ± 1.2 years) men, where the 12 students, constituted the control untrained group. All subjects performed a cycling exercise test to exhaustion. During exercise physiological characteristics: as heart rate (HR), oxygen uptake (VO_2), minute ventilation (V_E) and respiratory exchange ratio (RER) were measured. Before, after the exercise and at 1h recovery, in all subjects blood samples were taken for determine: total leukocyte count (WBC), leukocyte subsets (neutrophils, lymphocytes, monocytes, basophils, eosinophils, number and %) and serum cortisol level. The results of this study indicated that in response to similar exercise- physiological strain, there were no significant difference in WBC counts, percentage subsets of leukocytes and cortisol level between the CF and UT men however, during recovery greater neutrophilia and systemic post exercise lymphopoemia occurred in the trained men.

INTRODUCTION

Muscular exercise represents a model of physiological strain associated with changes in hormonal and immunological milieu depend on exercise intensity, type (aerobic, anaerobic), an individual fitness level and gender. In many cases responses to exercise stress have many similarities with clinical stressors [1,15]. Acute exercise affects the number and the function of circulating cells of both innate and adaptive immune system [21] and changes the release and concentration of numerous hormones, including norepinephrine, epinephrine, growth hormone (GH), cortisol, β -endorphin and adrenocorticotrophic hormone (ACTH), [4]. Several of these stress-induced hormones elicit substantial changes in total number and relative proportions of blood leukocytes [2]. Cortisol exerts on circulation leukocytes with a time lag and do not play a major role in excessive exercise leukocytosis [19] but may be responsible for systemic post exercise lymphopoemia and neutrophilia [19].

There are some evidences that show athletes during the heavy training are more susceptible to upper respiratory infection than sedentary men, due to deteriorated function of the immune system and decreased protection of the body against foreign and pathogenic

factors [6,16]. The effects of chronic exercise training on the cells of immune system are not completely known. Horn et al. [9] analyzed blood samples of 1000 elite male athletes in 14 different sports disciplines. The authors have found the lowest total WBC and neutrophil counts in athletes who were engaged in individual, highly aerobic sports such as cycling and triathlon. The highest WBC counts they observed in team sports, namely rugby and water polo, whereas no substantial changes in total or differential WBC count observed in strength trained individuals [10]. Changes in white blood cells number and their functions in sportsmen's represent an adaptive response of the immune system to exercise and indicate some differences in the body protection against pathogens between sports.

THE AIM OF THE WORK

Taking into account the main purpose of the present study was to examine the effect of a cycling exercise test performed to exhaustion on blood cortisol level and changes in total number and relative proportions of leukocytes in peripheral blood in untrained and CrossFit trained men.

MATERIAL AND METHODOLOGY

Thirty-one healthy men were recruited to participate in this study. The 12 subjects were the control group (n=12) consisted of untrained men (university students, UT) who participated only 2 times a week in physical education classes included in the university curriculum and 19 men were the trained group (CF). The study group (n=19) was consisted of CrossFit trained men with a period of training experience (5 ± 1.2 years). Study protocol was approved by the Ethical Committee at the Academy of Physical Education in Katowice and conducted in accordance with the Declaration of Helsinki. All subjects were familiarized with the exercise and measurement protocols and equipment prior to proceeding. The selected anthropometric and physiological characteristics of the subjects are presented in Table 1.

Exercise test: Each exercise trial was conducted at the p.m. at the same time of day in all exercise tests. During the test subjects performed a graded exercise test until volitional exhaustion (on a cycle ergometer -Ergomedic 839E, Monark Exercise AB, Sweden). The exercise started from initial load of 0 W for all subjects and than intensity of exercise increased every three minutes by 40 W at to individual maximal work loads. Exercise stopped when the participant could no longer maintain exercise at the given intensity.

Measurements: Before, after cessation of exercise and at 1h recovery blood samples from the a antecubital vein were taken for determine: in the blood: total leukocyte number (with division of leukocytes into 5 fractions: neutrophils, lymphocytes, monocytes, eosinophils, basophils, using automated hematology analyzer, Sysmex XE-2100 Corporation), in serum: total protein concentration (using the biuret method by means of the Hitachi 917) and cortisol level (measured using radioimmunoassay kit for the quantitative determination of cortisol in human serum CORT-CT2, Cisbio Bioassays, France) The sensitivity for cortisol assays was: $6,6 \text{ nmol} \cdot \text{l}^{-1}$.

At rest and during exercise were measured: heart rate (Polar S410; Polar Electro, Finland), oxygen uptake (VO_2) and respiratory exchange ratio (RER) using an open-circuit respiratory analyzer (Metalyzer 3B, Cortex, Germany). The metabolic rate was determined from VO_2 and RER via indirect calorimetry method, and expressed in watts (W/AD), AD-surface area calculated according DuBois DuBois [3] formula. The metabolic rate, or physical metabolic cost of the exercise, was calculated using a validated formula Gagge and Nishi [5].

Changes in plasma volume were calculated from the changes in serum total protein concentrations determined before, after cessation of exercise and at 1h recovery [8]. Due to the changes in plasma volume as a response to exercise, cortisol levels after the exercise and at 1h recovery were corrected according to Kraemer's methodology [11].

Statistical analysis: Statistical analysis was done using IBM SPSS Statistics version 10 (New York, USA). Data are presented as mean \pm standard error (SE). Separate analyses were performed to determine the difference for anthropometric and physiological characteristics and for total WBC and subsets of leukocytes. To compare differences between the CF and UT group t-tests for independent samples were used for data with a normal distribution and the Mann-Whitney U test for data which was not normally distributed (anthropometric and physiological characteristics). One way analyze of variance with repeated measures (ANOVA) was used to analyze the effects of group on variables as WBC number and leukocyte subsets and blood cortisol level. If a significant effect was found the statistical power (post-hoc Turkey's test) was calculated. The level of significance was set at $p \leq 0.05$. Pearson correlation coefficients were used to identify relationships between VO_{2max} and WBC count as well as between serum cortisol level and total WBC number and fractions of blood leukocytes.

RESULTS

Physical fitness and anthropometric subject's characteristics are presented in table 1.

Table 1. Subject's characteristics

Characteristics	Trained CF	Untrained
N=	19	12
Age (years)	23.27(0.79)	22.21(0.64)
Body height (cm)	179.91(1.62)	180.47(1.30) $p=0.06$
BM (kg)	78.95(2.20)	72.97(1.77) **
A_D (m^2)	1.98(0.03)	1.91(0.03) *
W_{max} (W)	304.44(9.99)	296.34(10.56)
Met_{max} ($W \cdot (m^2)^{-1}$)	460.17(18.64)	544.11(14.99) **
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	41.91(1.68)	51.23(1.35) ***
RER_{max}	1.21(0.02)	1.14(0.01)
VO_{2max} ($l \cdot min^{-1}$)	3.32(0.16)	3.78(0.13)
HR_{max} ($beats \cdot min^{-1}$)	186.54(2.12)	182.19(1.71)

Values are presented as mean \pm (SE); SE-standard error; Body mass –BM; Body surface area - A_D ; Maximal oxygen uptake - VO_{2max} ; Maximal heart rate - HR_{max} ; Respiratory exchange ratio-RER; Metabolic rate –Met; Maximal power of work W_{max} ; * significant difference between trained and untrained group (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$)

The baseline concentration of cortisol and values of total blood leukocytes (WBC), lymphocytes, neutrophils, monocytes, basophils and eosinophils, were within normal clinical range (Table 2, Fig. 1 and 2).

Exercise induced substantial leukocytosis ($p < 0.001$) in all subjects. The greatest post exercise increase was observed in neutrophils (increase of 53.72(4.42)% in the CF and of 55.6(4.69)% in the UT group) and lymphocytes (increase of 4.72(1.40)% in the CF and of 6.65(1.48)% in the UT group) count (Fig 1. and 2).

All dependent variables showed effect for time ($p < 0.05$) following the exercise, indicating physiological stress following the performed exercise test. Total WBC count decreased during recovery, but the percentage contribution of neutrophils as subsets of leukocytes in peripheral blood significantly increased. The percentage contribution of lymphocytes, monocytes, basophils, eosinophils in total leukocyte count dropped below baseline level at 1h recovery (leukogram) (Table 3). The changes in peripheral blood leukocytes are presented in Table 3.

Table 2. Changes in total blood leukocyte number, serum cortisol level (corrected for changes in plasma volume), and changes in plasma volume (PV) during experiment in the untrained and trained men

	Trained CF		Untrained
WBC [$10^9/L$]			
$F_2=167.8$ $p=0.00$	Rest (A)	6.14(0.39)	6.13(0.43)
	Exercise (B)	9.59(0.56) ^{B-A}	9.36(0.61) ^{B-A}
	Recovery (C)	5.90(0.39)	5.73(0.42)
Cortisol [nmol/L]			
$F_2=0.93$ $p=0.39$	Rest(A)	352.32(25.22)	325.07(27.36)
	Exercise (B)	393.82(32.62)	321.66(35.38)
	Recovery (C)	390.26(26.41)	353.61(28.65)
ΔPV [%]			
	Exercise (A-B)	-9.12(0.86)	-7.99(0.92)
	Recovery (C-A)	+2.72(1.25)	+2.54(1.36)



F_1 main effect for group, F_2 effect for time, F_3 interaction between effect for group x time; A-B represents significant difference between rest and exercise, A-C represents significant difference between rest and recovery

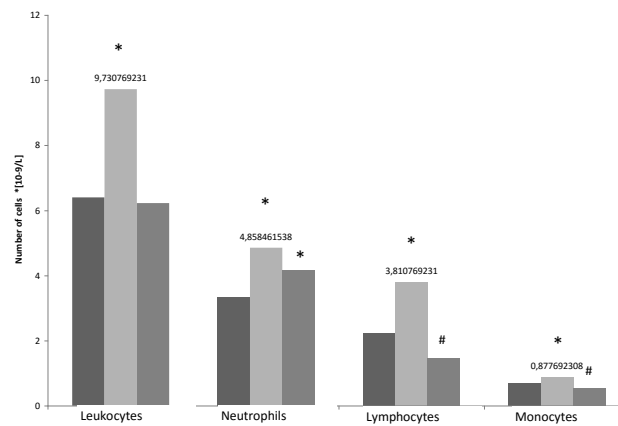


Fig. 1. Changes in total and subsets of blood leukocytes count measured before (rest), after the exercise, and at 1h post exercise in the CrossFit trained men, * $p<0.05$; ** $p<0.01$; *** $p<0.005$ exercise period significantly different to baseline, # $p<0.05$; ## $p<0.01$; ### $p<0.005$ - recovery period significantly different to baseline

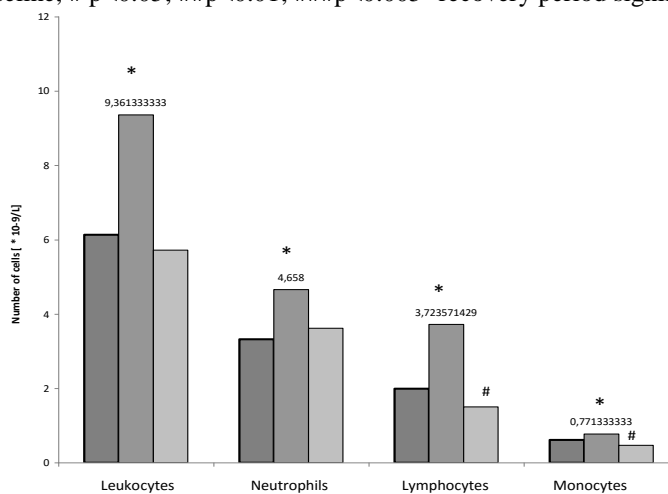


Fig.2. Changes in total and subsets of blood leukocyte count measured before (rest), after the exercise, and at 1h post exercise the untrained men
* $p<0.05$; ** $p<0.01$; *** $p<0.005$ exercise period significantly different to baseline, # $p<0.05$; ## $p<0.01$; ### $p<0.005$ - recovery period significantly different to baseline

Table 3. Percentage contribution of lymphocytes, neutrophils and monocytes, basophiles, eosinophils in total leukocyte count: at rest (A), immediately after exercise (B) and at 1h recovery (C) in the untrained and trained men

		Trained CF		Untrained	
Neutrophils, [%]					
F ₂ =74.85 p=0.00	Rest (A)	49.99(1.71) ^{A-C}	-	53.04(1.86)	
	Exercise (B)	48.41(1.78)	-	48.94(1.94) ^{B-C}	↑
	Recovery (C)	63.46(1.87) ^{C-B}	-	62.42(2.03) ^{C-A}	
Lymphocytes, [%]					
F ₂ =71.13 p=0.00	Rest (A)	35.92(1.60)	↑	33.26(1.74) ^{A-B}	↑
	Exercise (B)	40.4(1.65) ^{B-C}	-	39.92(1.81) ^{B-C}	
	Recovery (C)	25.89(1.56) ^{C-A}	-	26.81(1.71) ^{C-A}	↓
Monocytes, [%]					
F ₂ =37.03 p=0.00	Rest (A)	10.92(0.62) ^{A-B}	-	10.04(0.67) ^{A-B}	
	Exercise (B)	8.94(0.45)	-	8.29(0.49)	↓
	Recovery (C)	8.61(0.49) ^{C-A}	-	8.27(0.53) ^{C-A}	
Eosinophils, [%]					
F ₂ =42.71 p=0.00	Rest (A)	2.71(0.53) ^{A-B}	-	3.18(0.58) ^{A-B}	
	Exercise (B)	1.87(0.47)	-	2.41(0.51)	↓
	Recovery (C)	1.71(0.48) ^{C-A}	-	2.15(0.52) ^{C-A}	
Basophiles, [%]					
F ₂ =11.04 p=0.00	Rest (A)	0.43(0.04) ^{A-C}	-	0.46(0.05)	
	Exercise (B)	0.37(0.05)	↓	0.44(0.05)	
	Recovery (C)	0.32(0.05)	-	0.37(0.05)	

F₁ main effect for group, F₂ effect for time, F₃ interaction between the group x time; A-B significant difference between rest and exercise, A-C significant difference between rest and recovery

Serum cortisol concentration did not increase significantly in response to exercise and remained unchanged during recovery in all subjects (Table 2). Changes in monocyte (r=0.55; p<0.05) and basophile (r=0.69; p<0.05) counts correlated significantly with serum cortisol (Δ CORT) level only in the CF men. There were no significant correlation between fitness level and total WBC in the trained and UT men.

DISCUSSION

The results of the present study indicate that there are significant anthropometric and aerobic fitness differences between the CrossFit trained and untrained men. The CrossFit trained men have a significantly lower aerobic fitness level in comparison with the UT men,

but the metabolic cost (Met) of the exercise work (at the similar maximal workload (W_{max}) in (Table 1) in the CF men was significantly lower in comparison with the UT men.

The exercise-induced physiological strain induced substantial leukocytosis in trained and untrained men. The increase of leukocytes to an acute exercise bout is very typical response [4], but changes a pattern of leukocyte subpopulations response may depend on type of exercise performed [11], time of measurements, an individual fitness level, sex and type of sports [14, 9]. Horn et al. [9] have show that more aerobically oriented sports tend to have lower WBC and neutrophil counts.

In the current study, cycling induced great neutrophilia, following exercise in both tested groups. The rise of the neutrophil, lymphocyte and monocyte counts usually starts during the first minutes of endurance type exercise, whereas immediately after cessation of the exercise neutrophil count continues to increase, whereas lymphocyte and monocyte values drop below the pre-exercise levels and they stay low for 2h after the exercise [11]. The results of the present study support earlier findings however at 1h recovery neutrophil count remained significantly greater in comparison to baseline (+13%) in the CF trained men (Fig. 1) but returned to baseline values in the UT group (Fig. 2). Acute exercise bout induces changes in leukocyte subsets already during and immediately after the exercise bout [19]. The increase in neutrophil count is caused by demargination due to shear stress and increase in catecholamine concentration levels. Cortisol also exerts effects on circulating leukocytes, interacts with catecholamines, but do not play a major role in acute exercise leukocytosis [19]. Cortisol may be responsible for sustained post-exercise lymphopenia and neutrophilia [17] and for monocytosis in peripheral circulation immediately after the exercise caused by the actions of catecholamines, especially epinephrine [21]. In response to exercise strain the substantial leukocytosis was detected in all subjects however only in the CF trained men post exercise monocyte and basophile counts were significantly correlated with cortisol concentration.

Neutrophils contributed in total leukocytosis are the most when compared to other leukocyte cells, and this finding is in agreement with the results from other studies [13, 14, 20]. The number of basophiles in peripheral circulation is very low but also this cell type seems to be responsive to exercise stress in the present exercise test. The leukocyte values return to resting level within 24 hours after the end of the exercise. [4, 18, 19].

In cross-sectional studies Kim et al. [10] shown the association between physical parameters, such as VO_2max , and leukocyte count and indicated that higher VO_2max values were associated with lower total or differential WBC counts. On the other hand, Natale et al. [14] detected no significant association, between the number of immune cells and improvement in VO_2max in sports. The present study show that the exercise (performed to exhaustion) affects the leukocyte number of similarly in untrained and crossFit trained men and aerobic fitness do not significant correlate to post exercise leukocytosis. In present study the blood samples obtained at 1h recovery after exercise demonstrated that homeostasis in total leukocyte count was achieved during short recovery but leukocyte subsets did not return to the baseline values in the CF and UT men.

In response to exercise the concentration of several hormones increases (e.g. GH, cortisol, catecholamines and testosterone) that have that have an immunomodulatory effect [7, 19]. In present study positive correlations between increase in cortisol concentration and monocytes and basophiles after the exercise were detected in the CF but not UT men.

IN CONCLUSION

The exercise bout elicits leukocytosis in the untrained and trained men. There was no significant difference in total WBC count, percentage subsets of lymphocytes and cortisol level in response to similar exercise- physiological stain between the untrained and CrossFit

trained men. Lesser response of neutrophils and lymphocytes to exercise-induced physiological strain was indicated in the UT than CF men. however, during recovery greater neutrophilia and systemic post exercise lymphopenia ($<1-1.5 \cdot 10^9/L$) occurred in the CF trained men.

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