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Changes in the Number of Leukocytes and Lymphocyte Subpopulations Induced by Exercise in Sedentary Young People

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The influence of physical exercise on the quantification of leukocytes and lymphocyte subpopulations has been studied. These cells were obtained from blood taken from eleven healthy young men and women who follow a sedentary life style, before and after vigorous exercise (running). The results indicate that physical activity increases the number of white blood cells and the concentrations of circulating lymphocytes. The proportion of T-lymphocytes, estimated as rosette forming cells with sheep red blood cells after cold incubation, is constant, whereas a corresponding increase in cells with receptors for C3b or Ig-Fc is also observed. The data indicate that physical activity leads to an irregular intravascular discharge in stored cells.

Key words: Lymphocytes, Physical exercise, T-Lymphocytes, C3-Receptor bearing cells, Ig-Fc receptor bearing cells.

The continuous recirculation of lymphocytes from the blood vascular compartments into the lymphoid tissues in now generally accepted as essential for the proper functioning of the immune system. Evidence is accumulating to suggest that the rate of ingression and egression from the blood vascular compartment is relatively rapid in man (4).

The basal level of circulating white blood cells (WBC) in man can be rapidly and substantially increased by physical activity which mobilizes cells normally stored in undetermined sites (1). Short periods of exercise result in a phase of lymphocytosis which is followed by a neutrophyl phase during prolonged exercise (14) and whose magnitude is proportional to the intensity of work and duration of exercise (7). Physical activity may lead to an alteration in the number and function of lymphocytes (5). Knowledge of these physiological variations in peri-

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pheral blood lymphocytes in healthy persons could be essential and an important basis for clinical diagnosis and therapy of possible alterations in lymphocyte functions.

The aim of this work was to study the sequence of changes that a strenuous exercise produces in the total number of WBC, lymphocytes and their subpopulations, in healthy young sedentary men and women.

Materials and Methods

Eleven healthy young men and women volunteers between 20 and 25 years of age, after having been informed of the protocol involved, were included in the study. Each experiment started at 9.30 a.m. with the subjects in a fasted and rested state. Each subject was asked to run until exhaustion, the total distance covered being between 1.5 and 4 km depending on the subjects' fitness. Peripheral venous blood samples were taken in plain, previously heparinized tubes immediately before and after exercise. All procedures followed on human subjects were in accordance with the ethical standards of the Committee on Human Experimentation.

The WBC concentrations and leukocyte formula were performed using standard methods.

The lymphocytes were isolated from heparinized blood samples by Ficoll-Hypaque (Flow) density gradient centrifugation (3). The lymphocytes were then washed in Hank's solution and adjusted to 6×10^6 cells/ml of medium. The viability was considered as 95 % of the cells as revealed by the trypan blue exclusion test.

The lymphocyte subpopulations were recognized by their ability to form rosettes with sheep red blood cells (SRBC) (Materiales y Reactivos S. A., Madrid, Spain) with different treatment using the method described by JONDAL *et al.* (10). Briefly, T cells were assayed by mixing treatment-free SRBC with lymphocytes and incubating them at 4° C overnight (E-binding cells). Lymphocytes with Fc receptors were counted as cells binding SRBC coated with the IgG fraction of rabbit anti-SRBC serum (Materiales y Reactivos S.A.), for which anti-serum in subagglutinating amounts for 30 min (EA-binding cells).

Lymphocytes with receptors for complement (C3) were identified as cells forming rosettes with SRBC-antibody-complement. For this purpuse the SRBC were treated for 30 min at 37 °C with rabbit anti-SRBC serum and then incubated in the same conditions with the human complement.

All tests were performed in duplicate and the reading was carried out under the phase contrast microscope, considering as rosettes all lymphocytes that bound three or more sheep red blood cells around them. A minimum of 300 were counted in each sample and the results were expressed as rosettes-forming lymphocytes of the total lymphocytes present, as well as absolute concentrations of each subpopulation in blood, which were calculated by multiplying the percentage of the subpopulation with the absolute lymphocyte concentration.

All data are expressed as the mean ± S.D. of the number of experiments indicated in the corresponding tables and figures.

In the statistical study, Student's *t* test was used for the comparison between two parametric samples (the normality of samples was confirmed by Shapiro and Wilk's test).

Results

White blood cell count and leukocytic formula. — The total WBC was increased immediately after exercise in men as well as women. The lymphocyte percentages were also significat, without appreciable

Table I. *Influence in teenage men and women of physical activity in total count and percentage of peripheral blood leukocytes.*
Each value is the mean ± S.D. of 11 experiments performed in duplicate.

Sex		Total Leukocytes (cells/mm^3)	Leukocyte formula (%)		
			Monocytes	Neutrophils	Lymphocytes
Men	Before exercise	5263 ± 621	5 ± 2	66 ± 2	30 ± 3
	After exercise	6952 ± 237*	5 ± 1 n.s.	50 ± 6*	46 ± 8*
Women	Before exercise	5437 ± 372	5 ± 3	69 ± 4	26 ± 3
	After exercise	6258 ± 475*	5 ± 2 n.s.	55 ± 4*	40 ± 3*

* $p < 0.001$.

alteration in the monocyte percentage after running (table I).

Lymphocytes. — The number of lymphocytes (cells/mm^3) increased significantly ($p < 0.001$) in men (3125 ± 250) and women (3100 ± 450) after exercise with relation before exercise (2000 ± 220 men and 2100 ± 310 women). However, no difference is practically appreciated in the date between both sexes.

Lymphocyte subpopulations. — Table II shows that the absolute concentration of the E-binding lymphocyte subpopulations, T-lymphocytes, in both men and women, increased significantly after vigorous exercise. However, the percentage of the lymphocyte subpopulations forming E-rosettes, the habitual way of indicating T-lymphocytes, does not show any differences in the values before and after exercise in both sexes. Lymphocytes with IgG-Fc receptors (EA-binding) appear significantly increased in percentage and absolute concentrations in men and women, and the cells with receptors for the C3b fraction of the complement, EAC-binding, in women is significantly increased after exercise. However, in the mean values these lymphocyte subpopulations were only increased significantly when the results were shown in the total number of cells/mm^3.

Discussion

These results show that after strenuous exercise such as running until exhaustion a marked increase in leukocytes is produced in sedentary young people of both sexes.

The number of WBC are in general agreement with other authors (1, 2, 8, 9, 12, 18), who found similar data, with

Table II. *Influence of physical activity on lymphocyte subpopulations in teenage men and women.*
Each value es the mean ± S.D. of 11 experiments performed in duplicate.

Sex		E-Binding		EA-Binding		EAC-Binding	
		Percentage (%)	Total (cells/mm^3)	Percentage (%)	Total (cells/mm^3)	Percentage (%)	Total (cells/mm^3)
Men	Before exercise	43±3	855±128	6±3	123±53	26±3	479±177
	After exercise	41±3 n.s.	1384±337*	17±5*	545±182*	28±2 n.s.	904±149*
Women	Before exercise	45±3	922±169	6±3	125±45	23±4	472±139
	After exercise	45±3 n.s.	1448±274*	18±6*	581±244*	28±5*	897±231

* $p < 0.001$.

some variations in the absolute WBC values caused, in part, by the blood sample collecting technique and also by the counting method. However, the relationship between leukocytosis and the duration and intensity of exercise is controversial. While STEEL *et al.* (15) found that a short period of vigorous recreational exercise can induce a substantially delayed leukocytosis, other (11, 13) found a direct correlation between intensity and exercise duration with leukocytosis. On the other hand, WELLS *et al.* (17) in well trained male and female runners, found no changes in leukocytes after a marathon race.

Physical activity can induce changes in the concentrations of total peripheral WBC's but the modification produced in the different leukocytic populations with exercise can also be known. Substantial information concerning the effect of exercise on the quantification of neutrophils and lymphocytes, principally in trained sportsmen has already been published. However, few reports have been carried out in subjects following a sedentary life, which represents the greater part of the population, and they are the best model for showing the effect of exercise on the defence system. For this reason we considered it very interesting to study the exercise-lymphocytes subpopulations relation in subjects of sedentary life.

We have observed a significant elevation in the number of lymphocytes and subpopulations with receptors for C3 as well as cells with receptors for Ig-Fc, in healthy young men and women with a sedentary life-style after performing very vigorous exercise, in agreement with other authors (9, 5, 8, 12, 18). On the other hand, the total number per mm^3 of T-lymphocytes is also significantly increased with exercise but their percentage, the habitual form of representing T-cells, is not altered. HEDFORS *et al.* (9) obtained a decrease in T-lymphocytes in healthy sedentary individuals, in contrast with others (4, 6) who observed an increase in this cell population, particularly T-suppressor cells, in teenage athletes, different subjets from our young people leading a sedentary life.

The origin of the increase in circulating leukocytes caused by exercise is obscure. As our results show an increase in lymphocytes with membrane markers, which indicate maturity in these cells, they suggest that exercise does not induce liberation of lymphocytes from bone marrow, from where immature cells originate, but from the lymphoid organs, which play an important role in the removal of these cells (16).

In this work an increase in adherent lymphocytes with C3 and Fc-IgG receptors can be seen. The mechanism responsible for this leukocyte demargination was partially delineated by CRARY *et al.* (6), who demostrated that epinephrine increases cyclic AMP release from endothelial cells to which marginated leukocytes are attached. The increase of AMP served to reduce the adherence of leukocytes to the endothelium, thus releasing them into the circulation. The increase in total T-lymphocyte populations could be due to the changes in molecular binding on the surface of the lymphocytes that become separated from the endothelial cells and probably enter the blood from lymphatic tissues (18).

Further studies are needed to determine how age, sex, workload and duration of exercise, and physical fitness might affect the magnitude of the leukocytosis induced by exercise and to elucidate these mechanisms.

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Resumen

Se estudia la influencia del ejercicio físico sobre la cuantificación de leucocitos y subpoblaciones linfocíticas, de sangre extraída a hombres y mujeres jóvenes de vida sedentaria, antes y después de realizar un ejercicio vigoroso (carrera). Los resultados indican que el ejercicio físico aumenta el número de leucocitos y la concentración de linfocitos circulantes. La proporción de linfocitos T, estimadas como células formadoras de rosetas con hematíes de carnero después de incubadas en frío, es constante, mientras que se observa un incremento en el número de células con receptores para la fracción C3b del complemento, así como para la fracción Fc de la IgG. Los resultados parecen indicar que la actividad física provoca una descarga intravascular de forma irregular en los leucocitos.

Palabras clave: Linfocitos, Ejercicio físico, Linfocitos T, Receptor celular C3, Receptor celular Fc.

References

1. Ahlborg, B. and Brohult, J.: *Acta Méd. Scand.*, 182, 41-54, 1967.
2. Anderson, K. L.: *J. Appl. Physiol.*, 7, 671-674, 1955.
3. Boyun, A.: *Scand. J. Clin. Lab. Invest.*, 97, 77-89, 1968.
4. Christensen, B. E., Jonsson, U., Mathe, R. and Tonder, O.: *Scand. J. Haematol.*, 20, 246-257, 1978.
5. Christensen, R. D. and Hill, H. R.: *Amer. Pediatr. Haematol. Oncol.*, 9, 140-142, 1978.
6. Crary, B., Hanser, S. L. and Borysenko, M.: *J. Immunol.*, 31, 1.178-1.181, 1983.
7. Gary, W. E. and Bryan, W. R.: *Phys. Rev.*, 15, 597-638, 1935.
8. Giménez, M., Mohan-Kumar, T., Humbert, J. C., Talance, N. D. and Buisine, J.: *Eur. Appl. Physiol.*, 55, 465-470, 1986.
9. Hedfors, E., Holm, G. and Ohnell, B.: *Clin. Exp. Immunol.*, 24, 328-335, 1976.
10. Jondal, M., Holm, G. and Wigzell, H.: *J. Exp. Med.*, 136, 207-215, 1972.
11. Moorty, A. V., and Zimmerman, S. W.: *Eur. J. Appl. Physiol.*, 38, 271-276, 1978.
12. Robertson, A. J., Rameser, K. C. R. B., Potts, R. C. *et al.*: *J. Clin. Immunol.*, 5, 53-57, 1980.
13. Shoenfeld, Y., Aloni, D., Keren, G. *et al.*: *Acta Haematol.*, 65, 108-113, 1981.
14. Steel, C., Evans, J. and Smith, M.: *Nature*, 207, 387-389, 1974.
15. Steel, J. M., Steel, C. M. and Johstone, F. D.: *Br. Med. J.*, 295, 135-136, 1987.
16. Sttolman, L. M. and Rosen, S. D.: *J. Cell. Biol.*, 96, 722-729, 1983.
17. Wells, C. L., Stern, J. R. and Hecht, L. H.: *Eur. J. Appl. Physiol.*, 48, 41-49, 1982.
18. Yu, D. T., Clements, P. J. and Pearson, L. M.: *Clin. Exp. Immunol.*, 28, 326-331, 1977.