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Effect of Submaximal Physical Exercise Performed by Sedentary Men and Women on Some Parameters of the Immune System

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Submaximal physical exercise by sedentary men and women on a bicycle ergometer for 60 min at 58 % (men) and 55 % (women) of VO2 max. was evaluated for its effect on the number of total leukocytes (WBC), lymphocytes, monocytes and neutrophils and the percentages of $CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$ lymphocyte subpopulations, as well as on IgA, IgG and IgM serum immunoglobulin levels as one measure of B-lymphocyte function. Blood samples were collected before, immediately after, and 15 minutes after physical exercise. Exercise did not significantly change the total number of WBC, lymphocytes, monocytes or neutrophils, or the percentages of $CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$ positive cells and the Ig A and Ig M immunoglobulins. However the serum level of IgG immunoglobulins immediately after exercise was significantly lower than both before ($p < 0.001$) and 15 min later ($p < 0.05$). No differences in the results between men and women were detected.

Key words: Physical exercise, Lympocyte subpopulations, IgA, IgG and IgM, Serum immunoglobulin levels, Cortisol.

Physical exercise may be responsible for changes in lymphocyte recirculation and in the regulation of immune processes (16, 21). Reduction of the lymphoproliferative response to mitogenic stimulation (9), changes in natural killer cell activity (11), phagocytic function (2, 4, 8, 22, 23, 28), and delayed rejection of implanted tumors (26) are among the altered responses. The relationships between physical exercise and susceptibility to infections or immune-dependent disorders have been an-

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alyzed for many years. However, data on how physical exercise affects lymphocytes are in part conflicting; as well as not directly comparable because of different protocols (3, 14, 26, 30) and heterogeneity of the groups of subjects investigated. On the other hand, very few studies have been carried out on moderate physical exercise in sedentary people.

The purpose of the present work was to examine the effects of a single, submaximal, precisely defined effort performed by sedentary young people on certain quantitative characteristics of the lymphocytes which appear in the circulation as a result of exercise. Blood leukocyte counts and lymphocyte subpopulations were evaluated by using specific monoclonal antibodies for lymphocyte surface antigens ($CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$ cells), as well as the IgA, IgG, and IgM immunoglobulins as one measure of B-lymphocyte function, before, immediately after and 15 min after submaximal physical exercise on a bicycle ergometer. Since glucocorticoids have been shown to be responsible for modifications in the immune system induced by exercise, changes in the serum cortisol level were also evaluated, in case that could give additional information with respect to the modifications induced by physical exercise on the immune parameters measured in this work.

Materials and Methods

Subjects. — Ten men and ten women, healthy young student volunteers between 20 and 24 years of age, after having been informed of the protocol involved, were included in the study. Their physical characteristics are in table I. The students who qualified for participation had to be physically inactive, having undertaken no exercise program during the previous 24 months. In addition, they had to be healthy, non-smokers, and non-alcoholic. The VO_2 max values were consistent with published data on sedentary men and women for this age range.

Physical exercise. — The experiment started at 8.30 a.m. with the participants in a fasted and rested state. Each subject exercised on a bicycle ergometer for 60 min at a work intensity of 120 W, which gave an oxygen uptake (VO_2) of 1.9 l/min (men) and 1.8 l/min (women) corresponding to 58 % and 55 % of VO_2 max in men and women respectively (1). Peripheral venous blood samples were drawn by antecubital venepuncture before, immediately after, and 15 min after exercise. Pulse was recorded immediately before and after exercise.

Total leukocyte (WBC) and differential counts. — These were estimated by standard procedures.

Lymphocyte separation. — The lymphocytes were isolated from heparinized blood samples by Ficoll-Hypaque (Flow) density gradient centrifugation (6). The lymphocytes were then washed in CFT solution (Flow) and adjusted to 6×10^6 cells $\times$ ml^{-1} of medium. Viability was taken to be 95 % of the cells as revealed by the trypan blue exclusion test.

Lymphocyte subpopulations. — Lymphocyte subpopulations were identified by monoclonal antibody and complement-mediated cytotoxicity, using $CD3^+$ (all peripheral T cells), $CD4^+$ (helper-inducer T cells, Th), $CD8^+$ (suppressor-cytotoxic T cells, Ts) and $CD19^+$ (all peripheral B cells) (Ortho reagents). Briefly, the lymphocytes were incubated with monoclonal antibodies and rabbit complement (Behring) for 30 minutes, at which point eosin and formol were added as vital stain and fixative, respectively. At least 300 cells were counted in order to determine the percentage of mortality following addition of each antibody. Results were discarded when cell mortality was

Table I. *Characteristics of exercise (60 min effort) and physical characteristics of the 22 year old subjects.* Each value is the mean ± S.D. of 10 men or 10 women.

	Weight (kg)	Height (cm)	H.R. (beats/min)		VO_2	
			Before	After	l/min	max %
Men	74.9 ± 8.9	174 ± 6	74 ± 10	104 ± 6	1.9 ± 0.2	58
Women	54.9 ± 5.4	160 ± 3	79 ± 10	111 ± 7	1.8 ± 1.0	55

higher than 5 % in the controls (complement only) (31).

Cortisol serum levels. — Cortisol levels were measured by radioimmunoassay with a commercial kit (Immunotech). All assays were carried out in duplicate. Quality controls were included in all sets of measurements.

Immunoglobulin serum levels. — IgA, IgG and IgM immunoglobulin serum levels were measured by MANCINI radial immunodiffusion (20).

Statistical analysis. — All the values are expressed as the means ± S. D. of the number of experiments, performed in duplicate, as indicated in the corresponding tables and graphs. In the statistical study the normality of samples was confirmed by Shapiro and Wilk's test. Student's *t*-test was used for comparison between parametric samples.

Results

Submaximal physical exercise in sedentary subjects did not produce significant changes in the number of leukocytes, lymphocytes, monocytes or neutrophils in either men and women.

The percentages of $CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$ positive cells did not show any differences in the values before,

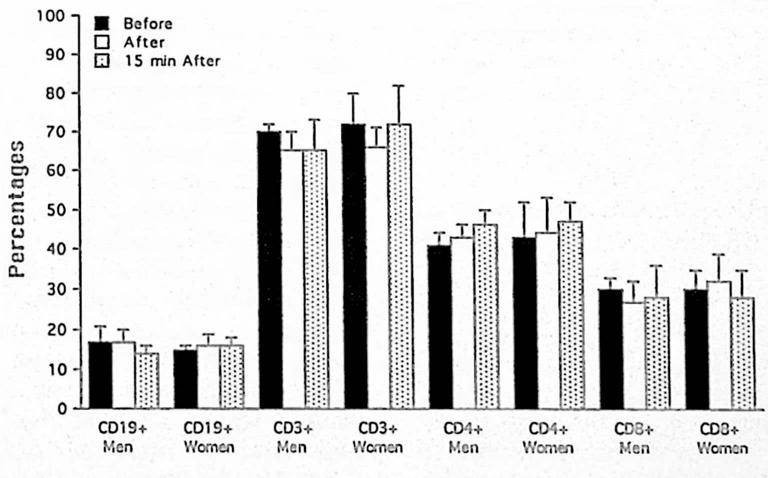

Fig. 1. *Changes in the percentages of $CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$ positive cells in men and women, before, immediately after and 15 min after submaximal physical exercise.*
Each column represents the mean ± S. D. of 10 experiments performed in duplicate.

Table II. *The effect of submaximal physical exercise on serum immonoglobulin levels (mg × 10 ml^{-1}) in untrained sedentary young men and women*
Each value is the mean ± S.D. of 10 men and 10 women. *** $p < 0.001$ with respect to value before exercise. * $p < 0.05$ with respect to the value before exercise. • $p < 0.05$ with respect to the value after exercise.

	IgA		IgG		IgM	
	Men	Women	Men	Women	Men	Women
Before exercise	221 ± 67	219 ± 50	1534 ± 319	1140 ± 298	118 ± 47	129 ± 32
After exercise	241 ± 98	238 ± 70	824 ± 161***	991 ± 85***	108 ± 42	110 ± 40
15 min after	256 ± 94	259 ± 69	1140 ± 279•	1300 ± 127*•	94 ± 20	100 ± 19

immediately after and 15 min after exercise in either sex (fig. 1).

Table II lists the results of the immunoglobulin concentrations. IgA and IgM immunoglobulins were not modified in these young sedentary men and women by physical exercise. However, the serum IgG levels fell immediately after exercise, and 15 minutes later the values were still lower than basal values. The immunoglobulin concentrations were statistically similar between the two sexes.

The results corresponding to serum cortisol concentrations are listed in table III. The submaximal exercise produced a significant fall in cortisol levels immediately after exercise in both the men and the women and 15 min after exercise in the women.

Table III. *The effect of submaximal physical exercise on serum cortisol levels (ng x ml^{-1}) in untrained sedentary young men and women.*
Each value is the mean ± S.D. of 10 men and 10 women. ***$p < 0.001$ with respect to the value before exercise. **$p < 0.01$ with respect to the value before exercise.

	Men	Women
Before exercise	238 ± 18	198 ± 60
After exercise	113 ± 23***	101 ± 34
15 min after	174 ± 132	104 ± 36**

Discussion

The present work evaluated the possible variations in the leukocyte and immunoglobulin concentrations in blood of healthy sedentary young adults who underwent bicycle exercise at 58 % and 55 % VO_2 max for men and women respectively, representing a submaximal exercise. The results showed no statistically significant differences in the total leukocyte, lymphocyte, monocyte and neutrophil concentrations either immediately after physical exercise or 15 minutes later. Neither were there significant changes in the percentages of $CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$ positive cells. These last results agree with other authors (5, 7, 18) who also obtained their results on untrained healthy volunteers performing non-acute exercise on ergometric bicycles. However, the results in the literature are very contradictory, since other workers have observed a fall in the $CD4^+/CD8^+$ ratio and an increase of the T cells ($CD3^+$), although in these cases after stressing exercise (10, 24, 30, 32). Likewise, published data generally indicate the appearance of significant leukocytosis after physical exercise (3, 19, 24, 28), although these authors also found these variations after subjecting the individuals to stressing exercise at maximum oxygen uptake. It seems reasonable to think, therefore, that, as the exercise rises in intensity, ever greater numbers of peripheral blood leukocytes are mobilised. In fact, an increase has been observed in the total leukocyte and T lymphocyte concentrations after exhausting exercise (3).

Cortisol has been shown to be responsible for the variations in leukocyte counts after physical exercise, although some authors (9, 13, 27) have been unable to establish a correlation between leukocyte numbers and cortisol levels. During intense exercise there are alterations in the blood concentrations of both endogenous catecholamines and endogenous cortisol which appear to be related to the leukocytosis (21). For these reasons, the modifications in serum cortisol levels were evaluated for the possible extra light that might be shed on the results. In the present case, with this type of exercise performed at 58 % (men) and 55 % (women) VO_2 max, there was a significant drop in cortisol levels immediately after exercise, the values tending to rise again 15 minutes later. Very strenuous acute physical exercise provokes markedly increased levels of cortisol (17), and, indeed, the plasma cortisol concentration is known to increase at work loads higher than 60 % of VO_2 max (21). However, the present results are consistent with those in the literature: other authors have found that at work loads below about 60 % of VO_2 max (21), plasma cortisol concentrations fall, because the rate at which cortisol is eliminated is higher during exercise than at rest, and the secretion rate at low workloads tends to be lower (or no higher) than at rest (21). Thus, the differences in the leukocyte counts in response to strenuous maximal exercise or moderate submaximal exercise could be in part attributed to the differences in cortisol secretion between the two types of exercise.

With respect to whether physical exercise produces changes in humoral antibody production, the results till now do not provide any clear data. Thus, KEAST *et al.* (16) indicated that the rudimentary information available suggests that *in vivo* humoral responses may not be modified to any great extent by exercise. HEDFORS *et al.* (15) found that, immediately after exercise *in vitro*, there is immune suppression of antibody synthesis, and PETROVA *et al.* (25) reported evidence for a reduction of both serum and secretory immunoglobulins which is related to increasing intensity of exercise work load. While, in the present work, there were no modifications found in IgA and IgM serum concentrations, there was observed to be a decrease in IgG immediately after exercise, tending to return to the basal level 15 min later. In general, it could be concluded that the humoral immune response is unchanged or decreases after exercise, depending on the isotype studied and the characteristics of the physical exercise. Clearly, as was noted by KEAST *et al.* (16), this area might be usefully investigated further.

No differences between men and women were found. In other aspects of the immune response, such as phagocytosis, some authors have found differences between men and women both in basal conditions (12, 28, 29) and in response to exercise. It seems reasonable to think, therefore, that sex differences in immune response to physical exercise could depend on the immune parameters being measured.

In summary, moderate submaximal physical exercise, where there is no increase in serum cortisol levels immediately afterwards, did not give rise to variations in leukocyte concentration or lymphocyte subpopulations. Moreover, this exercise provoked a decrease in serum IgG levels immediately afterwards. No sex differences were found.

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Resumen

Se estudia el efecto de un ejercicio submáximo, realizado por individuos sedentarios (58 % y 55 % VO_2 max en hombres y mujeres, respectivamente), sobre el número total de leucocitos (WBC), linfocitos, monocitos y neutrófilos, y sobre el porcentaje de subpoblaciones de linfocitos $CD3^+$, $CD4^+$, $CD8^+$ y $CD19^+$. Como medida de la funcionalidad de los linfocitos B se cuantifican los niveles séricos de las inmunoglobulinas IgA, IgG e IgM, con muestras de sangre obtenidas antes, inmediatamente después y tras 15 minutos de la finalización de la prueba ergométrica. El ejercicio submáximo no produce cambios significativos en el número de leucocitos (WBC, linfocitos, monocitos y neutrófilos), en el porcentaje de $CD3^+$, $CD4^+$, $CD8^+$ y $CD19^+$, ni en la concentración de IgA e IgM. Sin embargo, los niveles séricos de IgG inmediatamente después del ejercicio son significativamente más bajos que los que se obtienen en condición basal ($p < 0{,}001$) y los que se obtienen tras 15 minutos de la finalización del mismo ($p < 0{,}05$). En ningún caso se observan diferencias significativas entre los valores obtenidos en hombres y en mujeres.

Palabras clave: Ejercicio físico, Subpoblaciones de linfocitos, IgA, IgG e IgM, inmunoglobulina sérica, Cortisol.

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