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## ORIGINAL ARTICLE

# Phagocytes may counteract the "open window" situation during a bout of moderate exercise performed by sedentary individuals: role of noradrenaline

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**Summary**

Changes in the blood number of peripheral lymphocytes, monocytes and granulocytes at different times (basal, at 7 min, and immediately after exercise) during moderate exercise (55% $VO_2$ max; 60 min) performed by sedentary men were investigated. These variations were also evaluated during a recovery period (45 min and 24 h after finishing the exercise), and were correlated with changes in catecholamines and cortisol. The T (total $CD3^+$, $CD4^+$, and $CD8^+$) and B ($CD19^+$) lymphocytes showed similar behaviour during exercise, although the changes were more marked in T cells: an increase 7 min after the start of exercise that returned to the basal values immediately after exercise, and then a decrease (below basal) 45 min later to return again to the basal values 24 hours after the beginning of the physical activity. However, this behaviour was not shown by monocytes ($CD14^+$) and granulocytes ($CD15^+$), which increased their number from the beginning of the exercise and reached their highest values immediately after finishing it. NK cells ($CD16^+$) showed an intermediate behaviour between lymphocytes and phagocytic cells, with the highest values during exercise and the lowest ones (below basal) 45 min after the end of the physical activity. The profile in the plasma concentration of noradrenaline during the study correlated mainly with the profiles in the number of phagocytic cells, especially of monocytes. No significant correlations were found with adrenaline and cortisol.

It is concluded that, as previously reported for intense exercise, a bout of moderate exercise performed by sedentary individuals provokes changes in the incidence of lymphocytes and NK cells according to the "open window" theory. Phagocytic cells may couteract the "open window" situation, with the mechanism involving noradrenaline.

*Keywords*: exercise – immunology – leukocytes – stress hormones

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## INTRODUCTION

Today the effects of exercise and stress are often regarded as indistinguishable and the principal hormones released in all situations of stress are believed to be responsible for the exercise-induced changes in the immune system. Indeed, the interactions between exercise and the immune system are now considered to be a model of stress (Khansari et al. 1990, Ortega Rincon 1994, Hoffman-Goetz and Pedersen 1994, Ortega 2003).

The migration and circulation of immune cells, such as lymphocytes, monocytes and granulocytes, are events that allow the immune system to reach the sites where the immune response must develop. This is especially important during exercise, in which the response is mediated by the "stress hormones". Since 1902 it has been known that exercise provokes leukocytosis (Larrabee 1902). It is probably caused by the combined effects of glucocorticoids and catecholamines, and may be a mere redistribution phenomenon (McCarthy and Dale 1988, Brenner et al. 1998).

Leukocytosis is generally observed after intense exercise, in which the relationship with stress hormones is more clearly documented. Nevertheless, the profile of release of stress hormones can differ according to the exercise intensity and duration. Thus, during moderate exercise it would also be important to evaluate the variations in the profile of the number of immune cells in relation to a physiological stress response. Neuroendocrine responses of phagocytes during intense exercise are different from those found in lymphocytes (Ortega 2003), and this stimulation of the phagocytes may counter some of the pronounced suppressive effects of exercise on lymphocytes (Ortega Rincon 1994), even in the "open window" situation during the recovery period (Pedersen and Bruusgaard 1995). It is also probable that the quantitative behaviour of phagocytes and lymphocytes may differ in response to moderate exercise.

The purpose of the present investigation was to evaluate the quantitative behaviour (number of cells in the blood) of each leukocyte subpopulation during and after an acute bout of moderate exercise (55% $VO_2$ max; 60 min) performed by sedentary people, and to study whether these changes are related to a physiological stress response.

## MATERIALS AND METHODS

*Subjects*

Ten healthy male volunteers aged between 20 and 22 years participated in the study after having been informed of the protocol. To qualify for participation, they had to be physically inactive having undertaken no exercise programme during the previous 24 months, healthy, non-smokers, and not heavy consumers of alcohol. The experiment was approved by the Ethical Committee of the University of Extremadura (Spain) according to the guidelines of the European Community Council Directives.

*Physical exercise*

The experiment started at 9.00 a.m. with the participants having fasted, and having been in repose for at least one hour. Each subject exercised on a cycle ergometer (Ergometrix mod. Ergo-800S) for 60 min at an exercise intensity of 120 W, which gave an oxygen uptake ($VO_2$) of 1.875 ± 0.115 l / min corresponding to 55 ± 5% of maximal aerobic power ($VO_2$ max).

*Blood extraction*

Peripheral venous blood samples were drawn by antecubital vein puncture (a catheter with a two-way valve and physiological serum dropper was introduced into the vein 1 hour earlier in order to avoid the psychological stress of blood collection). Blood extraction was carried out before (basal), at 7 min after the start of the exercise, immediately after exercise, and 45 min and 24 hours after finishing the exercise.

*Quantification of immunocompetent cell number in blood*

The quantitative determination of lymphocytes, granulocytes, monocytes and NK cells was carried out by flow cytometric analysis. The "Immunocount" test (Cytognos) was used to quantify the immune cells in total blood anticoagulated with EDTA. The monoclonal antibodies anti- CD3, CD4, CD8, CD14, CD15, CD16 and CD19 were used (Ortho).

*Study of the variations in the stress hormone plasma concentrations*

Changes in the blood concentrations of cortisol and catecholamines (adrenaline and noradrenaline) were studied before exercise (basal), 7 min after the start of exercise, just after exercise, and 45 minutes and 24 hours later. For the cortisol assay, plasma was obtained by centrifuging (300 *g* for 20 min) 1 ml of anticoagulated blood from each participant. Cortisol was assayed by radioimmunoassay (R.I.A., DRG Instruments GmbH). For the catecholamine assay, 40 µl of a stabilizing solution (900 mg of EGTA and 700 mg of glutathione in 10 ml of 0.1 mol/l NaOH) was added to 2 ml of each blood sample before separation of the plasma. The plasma was then isolated by centrifugation as before. All plasma samples were stored at –30ºC until assay. Catecholamines were assayed by HPLC with electrochemical detection (Coulochem II), using a $C_{18}$ column (Waters), at a working potential between 450 and 660 mV, a 1 ml/min flow rate, and a pressure of at most 200 bar (Ortega Rincón et al. 2001). An internal standard (dihydroxybenzylamine) was used to allow the determination of losses during assay (Chromosystems Instruments and Chemical). After injection of 20 µl of the final processed sample, a

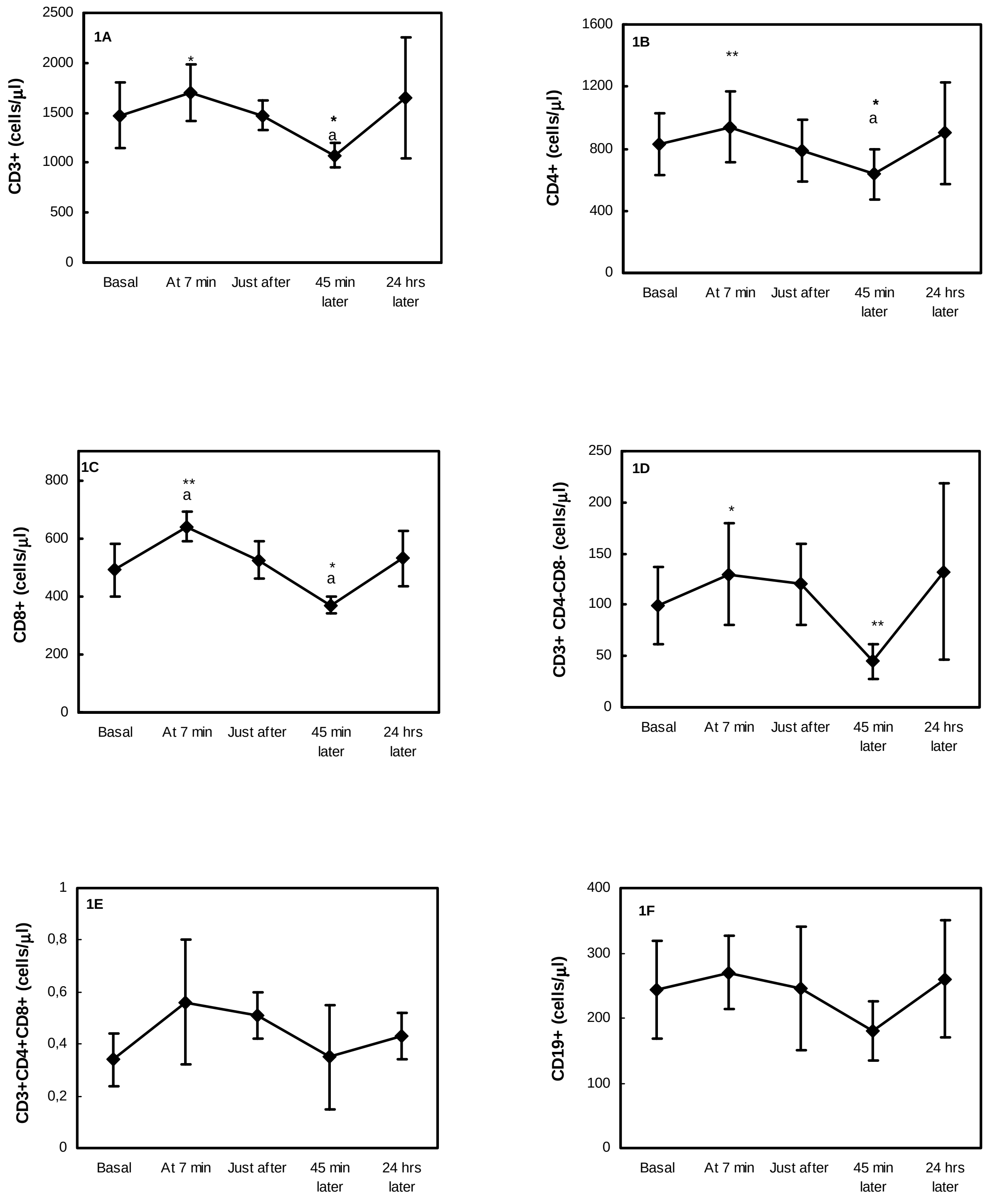


Fig. 1. **Changes in the number of the different lymphocyte subpopulations during and after moderate exercise.** Each value is the mean ± SD of 10 individuals performed in duplicate. * statistically significant as compared to the basal values; [a] statistically significant versus the other values (ANOVA statistical study)

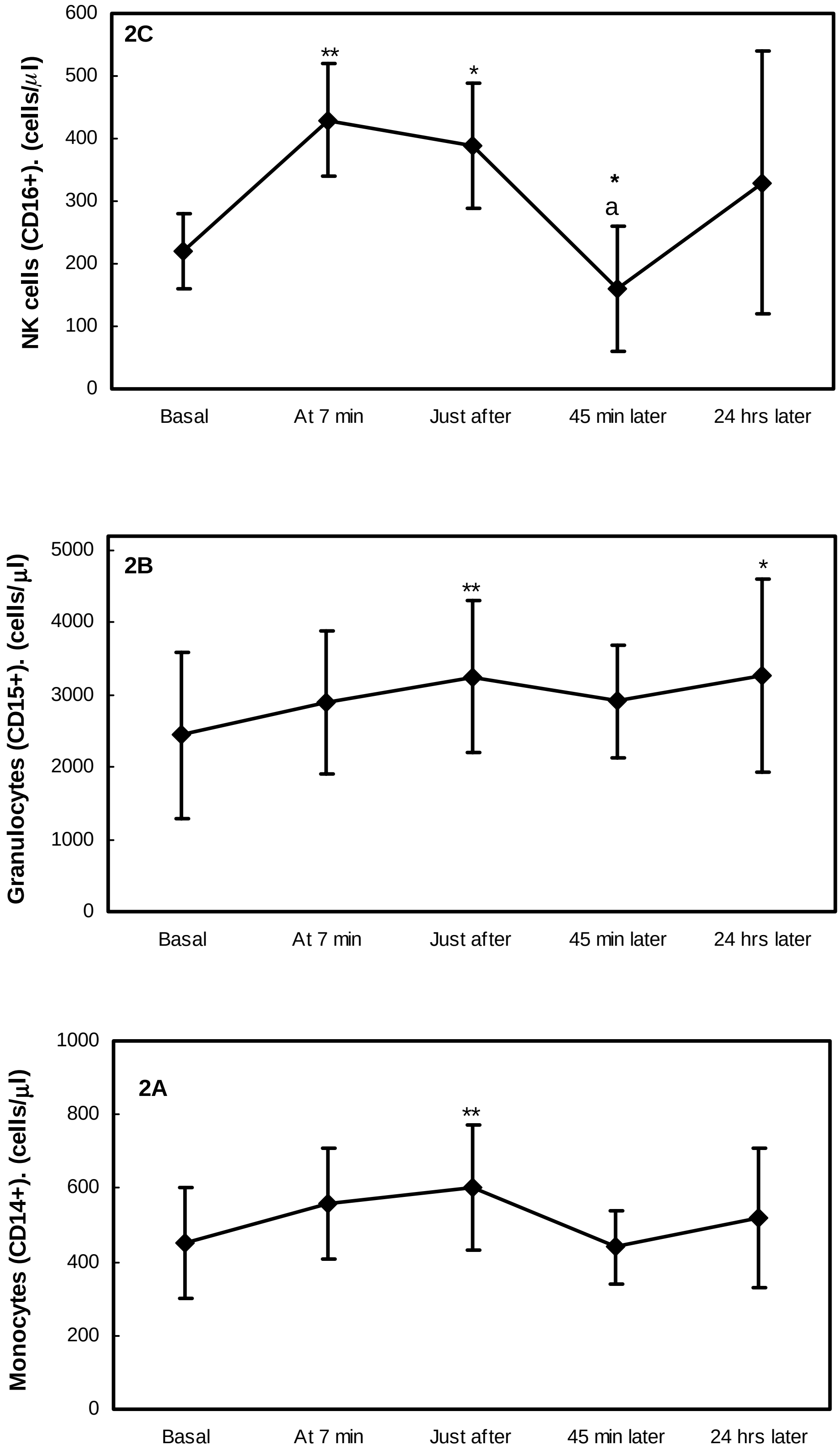


Fig. 2. **Changes in the number of monocytes (A), granulocytes (B) and NK cells (C) during and after moderate exercise.** Each value is the mean ± SD of 10 individuals performed in duplicate. Symbols as in Fig 1.

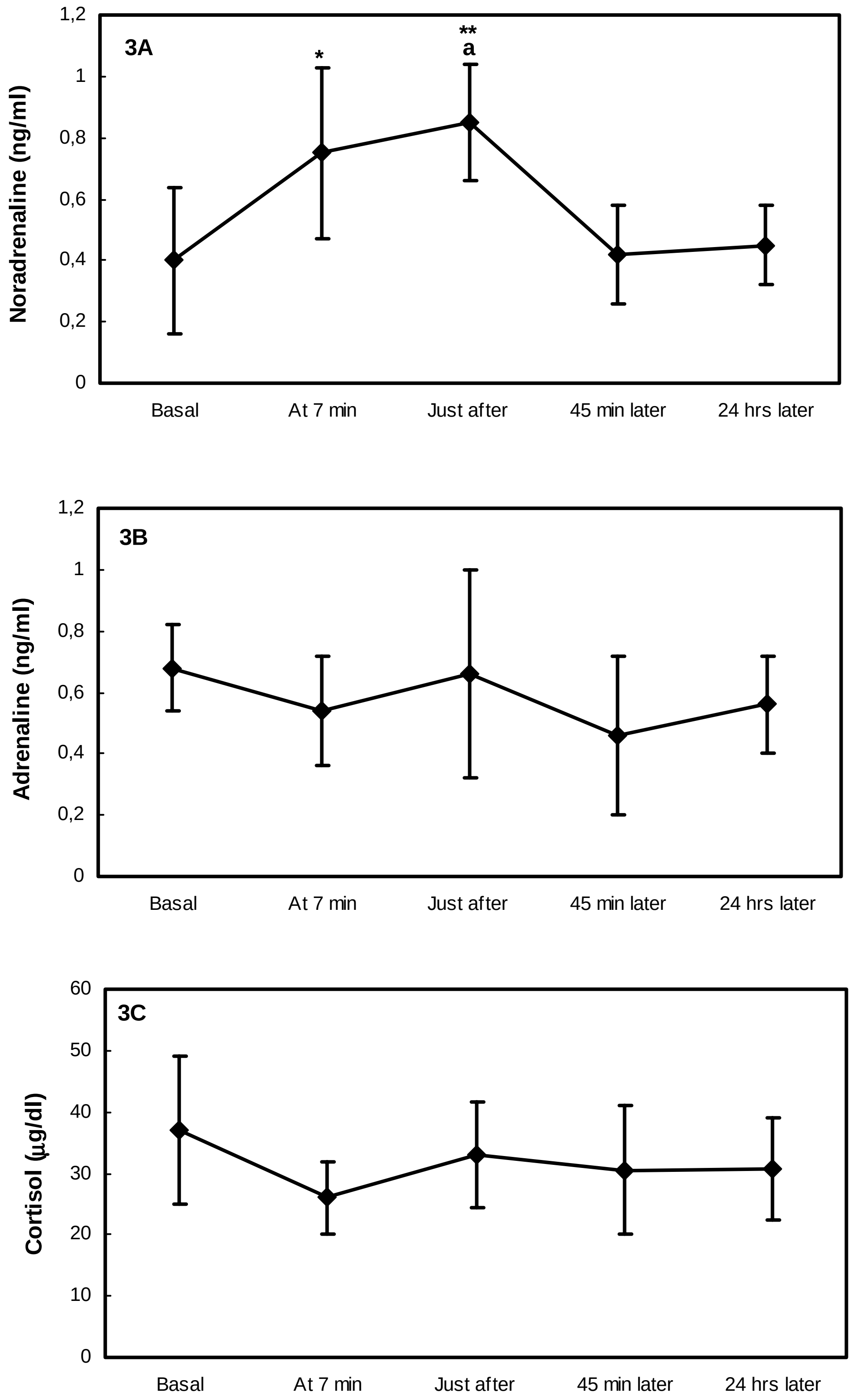


Fig. 3. **Changes in the blood concentrations of noradrenaline (A), adrenaline (B), and cortisol (C) during and after moderate exercise.** Each value is the mean ± SD of 10 individuals performed in duplicate. Symbols as in Fig. 1.

chromatogram was obtained in which one peak was observed at a lag of 4 min, corresponding to noradrenaline, and another at 5 min, corresponding to adrenaline.

*Statistical study*

The variables were normally distributed. The Student *t*-test was used to compare the differences between each value at the different times and the basal values. The parametric ANOVA Scheffe *F*-test was used to compare differences between all the values at different times at the significance level 2$\alpha$. Correlations were determined by multiple regression.

## RESULTS

Figure 1 shows the results corresponding to the variations in lymphocytes. With the Student *t*-test analysis, it was found that after 7 min of the start of the exercise, total $CD3^+$ lymphocytes (statistically significant; Fig. 1A) and $CD4^+$, or $CD8^+$ lymphocytes (statistically significant; Fig. 1B and 1C) increased their number with respect to the basal values. With the ANOVA study, T-lymphocytes ($CD3^+$, $CD4^+$ and $CD8^+$) showed the lowest number (statistically significant with regard to the other values) 45 min after finishing the exercise. No significant change was found in the $CD4^+/CD8^+$ lymphocyte ratio. $CD3^+$ $CD4^-$ $CD8^-$ cells also showed a statistically significant increased number at 7 min and a decreased (statistically significant) number 45 min after finishing the exercise relative to the basal values (Figure 1D). No significant variations induced by the exercise were found in the number of $CD3^+$ $CD4^+$ $CD8^+$ (Figure 1E). The profile of the number of B-lymphocytes ($CD19^+$ cells) was quite similar to those of the T-lymphocytes, although without significant variations between the different times (Figure 1F). No change was found in the T/B lymphocyte ratio.

Results corresponding to phagocytes and NK cells are shown in Figure 2. The profiles of the number of monocytes ($CD14^+$) and granulocytes ($CD15^+$) were quite similar during the exercise, with the highest values immediately after exercise (statistically significant with respect to the basal values). Unlike the behaviour of the lymphocytes, the number of phagocytes did not decrease during the recovery period. NK cells ($CD16^+$) also increased in number at 7 min after the start (statistically significant as compared to basal values) and immediately after exercise (statistically significant). The ANOVA statistical study showed the lowest values (statistically significant with respect to the other values) 45 min after finishing the exercise (Figure 2C).

Finally, the profiles of the blood concentration of catecholamines and cortisol are shown in Figure 3. No significant changes were found in the concentration of adrenaline (Figure 3B) and cortisol during exercise (Figure 3C). However, the blood concentration of noradrenaline increased at 7 min after the start of the exercise (statistically significant as compared to basal values) until immediately after finishing the exercise (statistically significant versus the other values), returning to the basal concentrations 45 min later.

No clear correlations were found between the behaviour of lymphocytes and the stress hormones. However, the variations during this study in the plasma concentration of noradrenaline correlated with the profile of the number of NK cells ($r^2$ = 0.8561), granulocytes ($r^2$ = 0.5967), and monocytes ($r^2$ = 0.8519) (Figure 4).

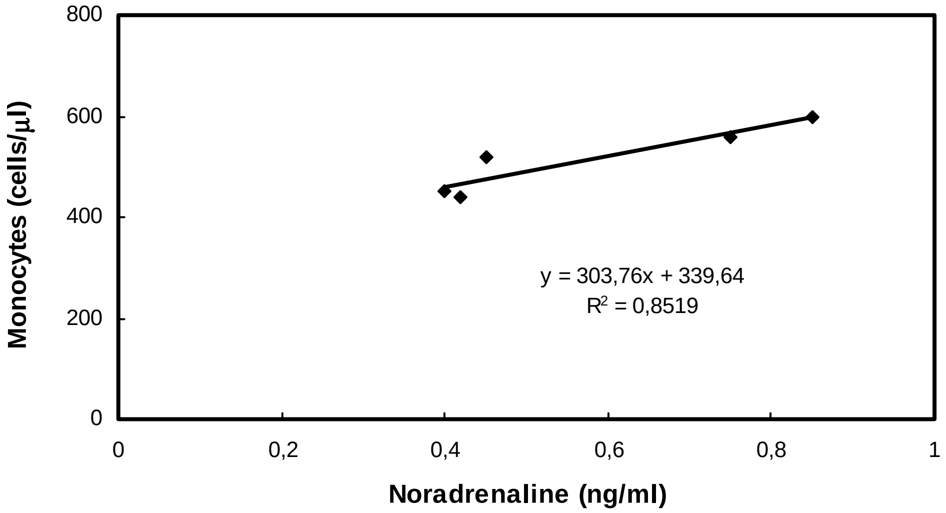


Fig. 4. **Correlation between noradrenaline and monocyte concentrations during and after moderate exercise**

**DISCUSSION**

It is generally accepted that when exercise is performed at an intensity higher than 75% of $VO_2$ max for 1 h or more, the neutrophil number increases during the exercise and continues to increase for several hours post-exercise. The lymphocyte number also increases during exercise, but falls below basal values following intense long-term exercise. Thus, in the recovery period of intense exercise (> 75% of VO2 max) of long duration (> 1 h), the number of the different lymphocyte subpopulations decreases (McCarthy and Dale 1988; Pedersen et al. 1998). In contrast, it is commonly reported that if the exercise is moderate, e.g. around 50% of $VO_2$ max, the lymphocyte count does not decline in the recovery period (Pedersen et al. 1998). However, in the present investigation it was found that moderate exercise (55% $VO_2$ max) also provokes changes in lymphocytes, even during the recovery period. The T and B lymphocytes showed a similar behaviour during exercise, but with more marked changes in T cells: an increase at 7 min after the start of physical exercise that returned to the basal values immediately after exercise, and then a decrease (below basal values) 45 minutes after finishing exercise to reach again the basal values 24 hours later.

The behaviour of monocytes ($CD14^+$) and granulocytes ($CD15^+$) was different from that of lymphocytes. They increased in number from the beginning of the exercise and reached the highest values immediately after finishing it, without any decrease during the recovery period. These results are in accordance with those reported for higher exercise intensities (Pedersen et al. 1998), although some studies have shown that the number of phagocytic cells also declines below baseline values in the recovery period after strenuous exercise (Wigernaes et al. 2001). NK cells ($CD16^+$) showed an intermediate behaviour between lymphocytes and phagocytic cells, with the highest values during exercise and the lowest ones (below basal) 45 min after the end of the physical activity. Although the intensity is responsible for the degree of increase in the number of NK cells, it has been reported that K cell activity increases when measured immediately after or during both moderate and intense exercise of a few minutes (Rhode et al. 1996, Pedersen et al. 1998). During the recovery period (45 min after exercising), our results showed a similar behaviour in NK cells to those reported following intense exercise of long duration (Pedersen and Ullum 1994): a decline in the number of NK cell below basal values.

The variations in lymphocytes and NK cells found during our model of moderate exercise performed by sedentary people also agree with the "open window theory" reported for intense exercise (Pedersen and Bruunsgaard 1995, Pedersen et al. 1998). However, the behaviour of the phagocytic cells (the first line of cell defense of the organism) may counteract the "open window" situation, as has also been reported previously for intense exercise (Ortega Rincón 1994). Studies performed in our laboratory demonstrated the hypothesis that a stimulated innate immune response mediated by phagocytic cells may counteract the immunosuppression in specific lymphocyte functions observed during and after strenuous exercise (Ortega Rincón 1994). This stimulation of the phagocytic cells is mediated by stress hormones (Forner et al. 1995, Ortega et al. 1997, Ortega 2003) which usually also induce immunosuppression in lymphocytes. The question now is whether the quantitative changes induced by the moderate exercise may also be mediated by the stress hormones. During our model of exercise, sedentary individuals did not show significant changes in adrenaline and cortisol concentrations. However, a significantly increased concentration of noradrenaline was found during exercise that returned to the basal values 45 min later. These results may agree with the idea that with moderate exercise the increase in leukocyte number is related mainly to the plasma noradrenaline concentration, but with more intense exercise the adrenaline concentration takes on a greater importance. The plasma cortisol concentration increases only with long-duration exercises, with minor changes of concentration in response to an acute time-limited exercise of 1 h (Galbo 1983, Brenner et al. 1998, Pedersen et al. 1998), as observed in our investigation.

In this study the profile of the plasma concentration of noradrenaline correlated with the variations in the number of NK cells ($r^2 = 0.8561$), neutrophils ($r^2 = 0.5967$) and, especially, of monocytes ($r^2 = 0.8519$). With the same moderate exercise protocol, neutrophils from sedentary individuals increased in phagocytic capacity (Ortega et al. 1993). Recently, we have also demonstrated that this stimulation is mediated by noradrenaline (Ortega et al. 2005). Thus, noradrenaline may mediate both the quantitative (number) and qualitative (function) stimulation of neutrophils during short-term acute moderate exercise, supporting the hypothesis that sympathetic nerve activity stimulates neutrophils (Nagatomi et al. 2000). This hypothesis seems also to be valid for monocytes/macrophages, because the changes in noradrenaline strongly correlated with the changes in monocytes, and it has also been reported that this neurohormone stimulates the phagocytic function of macrophages (García et al. 2003).

In conclusion, moderate exercise (55% $VO_2$ max for 60 min) performed by sedentary people induces changes in all leukocyte subpopulations. As reported for intense exercise, the variations in lymphocytes and NK cells is consistent with the“open window” theory. However, the behaviour of the phagocytic cells (neutrophils and monocytes) may counteract this “open window” situation under the mediation of noradrenaline.

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## REFERENCES

Brenner I., Shek P.N., Zamecnik J., Shephard: R.J.: Stress hormones and the immunological responses to heat and exercise. Int. J. Sports Med. 19:130–143, 1998.

Forner M.A., Barriga C., Ortega E.: A study of the role of corticosterone as a mediator in exercise-induced stimulation of murine macrophage phagocytosis. J. Physiol. 488:789–794, 1995.

Galbo H.: Hormonal and Metabolic Adaption to Exercise. Thieme Verlag, New York 1983.

Garcia J.J., Sáez M.C., De la Fuente M., Ortega E.: Regulation of phagocytic process of macrophages by noradrenaline and its end metabolite 4-hydroxy-3-metoxyphenyl-glycol. Role of - and -adrenoreceptors. Mol. Cell. Biochem. 254:299–304, 2003.

Hoffman-Goetz L., Pedersen B.K.: Exercise and the immune system: a model of the stress response? Immunol. Today 15:382–387, 1994.

Khansari D.N., Murgo A.J., Faith R.E.: Effect of stress on the immune system. Immunol. Today 11:170–175, 1990.

Larrabe R.C.: Leucocytosis after violent exercise. J. Med. Res. 7:76–82, 1902.

McCarthy D.A., Dale M.M.: The leukocytosis of exercise. A review and model. Sports Med. 6: 333–363, 1988.

Nagatomi R., Kaifu T., Okutsu M. et al.: Modulation of the immune system by the autonomic nervous system and its implication in immunological changes after training. Exerc. Immunol. Rev. 6:54–74, 2000.

Ortega E.: Neuroendocrine mediators in the modulation of phagocytosis by exercise: physiological implications. Exerc. Immunol. Rev. 9:70–93, 2003.

Ortega E., Collazos M.E., Maynar M., Barriga C., De la Fuente M.: Stimulation of the phagocytic function of neutrophils in sedentary men after acute moderate exercise. Eur. J. Appl. Physiol. 66:60–64, 1993.

Ortega E., Forner M.A., Barriga C.: Exercise-induced stimulation of murine macrophage chemotaxis: role of corticosterone and prolactin as mediators. J. Physiol. 498:729–734, 1997.

Ortega E., Marchena J.M., García J.J., Barriga C., Rodríguez A.B.: Norepinephrine as a mediator in the stimulation of phagocytosis induced by moderate exercise. Eur. J. Appl. Physiol. 93: 714-718, 2005.

Ortega Rincón E.: Physiology and Biochemistry. Influence of exercise on phagocytosis. Int. J. Sports Med. 15:172–178, 1994.

Ortega Rincón E., Marchena J.M., García J.J. et al.: Phagocytic function in cyclists: correlation with catecholamines and cortisol. J. Appl. Physiol. 91:1067–1072, 2001.

Pedersen B.K., Bruunsgaard H.: How physical exercise influences the establishment of infections. Sports Med. 19:393–400, 1995.

Pedersen B.K., Ullum H.: NK cell response to physical activity: possible mechanism of action. Med. Sci. Sports Exerc. 26:140–146, 1994.

Pedersen B.K., Rhode T., Ostrowski K.: Recovery of the immune system after exercise. Acta Physiol. Scand. 162:325–332, 1998.

Rohde T., MacLean D.A., Hartkopp A., Pedersen B.K.: The relationship between plasma glutamine level and cellular immune responses in relation to triathlon race. Eur. J. Appl. Physiol. 74:428–434, 1996.

Wigernaes I., Hostmark A.T., Stromme S.B., Kierulf P., Birkeland K.: Active recovery and post-exercise white blood cell count, free acids, and hormones in endurance athletes. Eur. J. Appl. Physiol. 84:358–366, 2001.