Acute Effects of Soccer Training on White Blood Cell Count in Elite Female Players

Alexandra A. Avloniti, Helen T. Douda, Savvas P. Tokmakidis, Alexandros H. Kortsaris, Evropi G. Papadopoulou, and Emmanouil G. Spanoudakis

Purpose: To investigate the acute changes in leukocyte number and cortisol after a single bout of soccer training. Methods: Ten elite female national-team soccer players and 8 nonathletes participated in the study. The duration of the exercise was 2 h, and it was performed at an intensity of 75% of maximal heart rate (HRmax). Blood samples were taken before, immediately after, and 4 h after a soccer training session to determine total white blood cells; the subsets of neutrophils, lymphocytes, monocytes, eosinophils, and basophils; and cortisol. At the same time, blood samples were obtained from nonathletes who refrained from exercise. Results: Data analysis indicated a significant increase in total white blood cells in the athletes postexercise ($P < .001$). The leukocytosis was still evident after 4 h of recovery (78% higher than the preexercise values), and there was a significant difference between athletes and nonathletes ($P < .001$). This leukocytosis was primarily caused by neutrophilia—there were no significant differences in lymphocytes after the end of exercise or between the 2 groups ($P > 0.05$). In addition, there was a statistically significant difference in cortisol concentration between athletes and nonathletes after the exercise ($P < .001$). Conclusion: These findings revealed that the single bout of soccer training at an intensity of 75% of HRmax induced leukocytosis without affecting the lymphocyte count in elite female athletes and probably the effectiveness of cellular components of adaptive immunity. Coaches should provide adequate time (>4 h) until the next exercise session. Key Words: immune, leukocytes, neutrophils, lymphocytes, cortisol

It is well recognized that exercise causes considerable changes to the number and function of immune cells in circulation. Nieman\textsuperscript{1} declared that a plethora of research worldwide has greatly increased our understanding of the relationship between prolonged, intensive exercise; the immune system; and host protection against viruses and bacteria. It is often claimed that elite athletes experience a greater incidence of illness and infection during periods of prolonged or intense training. On the other hand, physically active members of the general public who...
undertake regular physical activity with moderate intensity feel healthier and suffer fewer illnesses.\textsuperscript{2} The concern for athletes, coaches, and sports-medicine scientists is to reduce the incidence of illness during intense training periods and competitions. According to the “open window” theory, exercise is associated with a transient but clinically significant depression of immune function that might last between 3 and 72 hours depending on the immune parameter measured, as well as the type, duration, and intensity of exercise. During this period viruses and bacteria might gain a foothold, increasing the risk of subclinical and clinical infection.\textsuperscript{3}

During and immediately after exercise the number of white blood cells in circulation increases. The magnitude of these changes appears to be directly related to exercise intensity and duration.\textsuperscript{4,5} During the postexercise period, there is a decline in the number of circulating lymphocytes to below resting values,\textsuperscript{6,7} whereas the circulating neutrophil count has been reported to increase, peaking several hours postexercise.\textsuperscript{5,8} This biphasic response is widely reported in the literature. In addition, lymphocytopenia has been observed before the cessation of a prolonged bout of intense exercise.\textsuperscript{9} Even though moderate-intensity or short-duration exercise might induce perturbations to circulating cell numbers for approximately an hour after the end of exercise, homeostasis might not be restored for several hours.\textsuperscript{10}

The accurate mechanisms by which leukocytes are released into the circulation during and after exercise have not been fully clarified.\textsuperscript{4} There is evidence, however, to implicate both catecholamines and cortisol as mediators of this process.\textsuperscript{5,11} It is proposed that catecholamines produced during exercise act to increase the ratio of circulating to noncirculating leukocytes, and cortisol is responsible for a second later phase of leukocytosis, mainly by promoting leukocyte release from the bone marrow.\textsuperscript{5}

Numerous studies show immunosuppression in endurance athletes; moreover, in team sports only a limited number of studies have investigated the acute effects of football play after matches\textsuperscript{12} or in response to field- or laboratory-based tests designed to stimulate the activity patterns and physiological demands of football in male athletes.\textsuperscript{13,14} To our knowledge, no study has been undertaken to examine the acute changes in the number of leukocytes after soccer training in elite female players in order to observe the clinical consequences occurring after an exercise session. Therefore, the purpose of the current study was to investigate the acute changes in the number of leukocytes and cortisol after a single bout of soccer training in elite female athletes.

**Methods**

**Subjects**

Ten elite female soccer players who were members of the Greek national team and 8 female physical education students who were not involved in competitive athletics participated in the study. All the subjects were healthy (not suffering from fever, asthma, or allergies), had no indication of musculoskeletal injury, were not taking any medication, and had not suffered from an infection or illness in the preceding 4 weeks. The study was approved by the university ethics committee. Participants were informed in detail about the experimental procedures and signed an informed-consent statement.
Anthropometric Measurements

Using the techniques described in the Anthropometric Standardization Reference Manual, height, body mass, and subcutaneous skinfold thickness were measured. Percentage body fat was assessed by using skinfold measurements of the triceps, suprailiac, abdomen, and thigh for athletes and nonathletes. Table 1 presents the mean and standard-deviation values of the subjects’ physiological characteristics.

Maximal Oxygen Uptake

A maximal-oxygen-uptake (VO$_2$max) test was performed on the treadmill. The initial speed was set at 8 km/h and was increased by 2 km/h every 3 minutes until exhaustion. Oxygen consumption was continuously monitored throughout exercise by an Oxycon Champion analyzer (IEC 601-1, Jaeger, Wuerzburg, Germany). The criteria used for the attainment of VO$_2$max were that the subjects had to reach both a plateau of VO$_2$ (<1.8 mL·kg$^{-1}$·min$^{-1}$) increase) with an increase in work rate and a respiratory-exchange ratio above 1.10. Heart rate was continuously monitored by a Polar S810 monitor (Kempele, Finland).

Exercise Protocol

The study was conducted at the beginning of the preparatory training phase before the Olympic Games in Athens. Changes in leukocytes and cortisol were investigated in response to a single bout of soccer training in elite female athletes. The training session consisted of warm-up (20 minutes) of jogging and calisthenics exercises; static stretching (10 minutes) of the gastrocnemius, quadriceps, hamstring, leg-adductor, and deep abdominal muscles; and 3 sets of 25 minutes of games with 5 minutes rest between sets. The latter part was organized as playing sessions and was focused on tactical aspects. Heart rate was recorded for each athlete during the entire length of the exercise session using a heart-rate monitor (Polar S810, Kempele, Finland). The duration of the exercise session was 2 hours, and it was

| Table 1 Physiological Characteristics (Mean ± SD) of Female Soccer Players and Control Group |
|-------------------------------------|-----------------|-----------------|
| Characteristics                    | Female soccer players (n = 10) | Control group (n = 8) |
| Age (y)                            | 23.8 ± 3.5       | 25.3 ± 3.0       |
| Training age                       | 12.4 ± 6.2       | —                |
| Height (cm)                        | 166.2 ± 6.7      | 170.3 ± 5.3      |
| Weight (kg)                        | 59.9 ± 7.2       | 59.2 ± 5.8       |
| Body fat (%)                       | 13.2 ± 1.0       | 16.1 ± 3.0       |
| Fat mass (kg)                      | 7.9 ± 1.2        | 9.5 ± 1.9        |
| Lean body mass (kg)                | 52.0 ± 6.2       | 49.7 ± 2.2       |
| VO$_2$max (mL·kg$^{-1}$·min$^{-1}$) | 55.8 ± 4.1       | 42.6 ± 4.2       |
| HRmax (bpm)                        | 195 ± 6          | 193 ± 5          |
performed at an intensity of 75% of maximal heart rate (as determined during the VO\textsubscript{2}max test).

**Blood Sampling and Analysis**

The first blood samples were drawn at rest after an overnight fast and refraining from exercise for 3 consecutive days for both athletes and the control group. Blood samples were taken for the athletes before exercise (8 AM), immediately after (12:30 PM), and 4 hours after the end of the session (4:30 PM) to measure total white blood cells; the subsets of neutrophils, lymphocytes, monocytes, eosinophils, and basophils; and cortisol.

At the same times (8 AM, 12:30 PM, and 4:30 PM), blood samples were obtained from nonathletes at rest, who had not participated in any physical activity, to examine possible diurnal variations. Furthermore, controls were asked to avoid any other possible physical or mental stressors during the day of blood sampling.

Blood samples were drawn from an antecubital vein with subjects in the seated position. Three milliliters of blood were collected into Vacutainer tubes containing ethylenediaminetetraacetic acid for leukocyte analysis, and 2 mL were placed in untreated Vacutainer tubes for analysis of cortisol. For hormonal analysis, the blood sample was centrifuged and the serum was stored at –80°C until analysis.

White blood cell count was measured by an automated hematology analyzer (Sysmex KX21, Kobe, Japan). Because cell numbers were determined in whole blood, corrections for changes in plasma volume were not made.\textsuperscript{12} Cortisol concentrations were analyzed in duplicate by an enzyme-linked immunosorbent assay (ELISA, DRG Diagnostics, Germany). Intracorrelation and intercorrelation coefficients were 4.5% and 8.2%, respectively. Because cortisol was determined in serum, changes in plasma volume were estimated using the method of Dill and Costill.\textsuperscript{17}

**Statistical Analysis**

A 2-way (Group × Time) analysis of variance with repeated measures was used to examine differences between means. A Bonferroni post hoc test was applied whenever significant differences were found for interaction or main effects. The accepted level of significance was set at \( P < .05 \). The results are presented as mean ± SD.

**Results**

There were no significant differences in leukocytes or cortisol between the athletes and the control group preexercise.

**White Blood Cells**

The significant interaction of group and time demonstrated a different pattern of total white blood cell counts over time between groups \( (F_{2,32} = 55.12, P < .001) \). In particular, white blood cells of soccer players increased 70% immediately after exercise \( (P < .001) \), whereas no time effect was observed in the control group \( (P > .05) \). Four hours after exercise white blood cells had further increased in the
athletes, demonstrating their highest values (8% as compared with immediately after exercise and 78% as compared with baseline values), whereas the values of the control group remained unchanged (Figure 1).

**Neutrophils**

The analysis of neutrophils data showed a significant interaction between group and time ($F_{2,32} = 70.83, P < .001$). Neutrophils were significantly elevated in athletes ($P < .001$) after exercise ($2.68 \pm 0.174 \times 10^9/L$ to $6.51 \pm 0.225 \times 10^9/L$) and remained elevated, reaching the highest value at 4 hours ($P < .001$) as compared with preexercise, whereas no changes were observed in the control group at any time point (Figure 2).

**Lymphocytes**

There was no significant interaction of group and time in lymphocytes ($F_{2,32} = 0.36, P > .05$). After exercise, the lymphocyte count decreased slightly by 6.3% ($P > .05$) in athletes and returned to preexercise values at 4 hours (Figure 2). No significant differences between soccer players and the control group were found after exercise ($P > .05$) at any time point.

![Figure 1](image.png)

**Figure 1** — White blood cells (mean ± SD) in athletes and controls at different time periods (pre: 8 AM; post: 12:30 PM; 4 h: 4:30 PM) after the athletes underwent a 2-h training session. ***Significantly different from athletes ($P < .001$). †††Significantly different from preexercise ($P < .001$).
Monocytes

The significant interaction of group and time ($F_{2,32} = 6.54$, $P < .01$) demonstrated a different pattern of monocytes between groups. In the exercise group, monocytes increased from $0.350 \pm 0.029 \times 10^9/L$ before exercise to $0.446 \pm 0.043 \times 10^9/L$ after the exercise bout ($P > .05$). The increase at 4 hours (41%) was significant ($P < .01$) compared with preexercise values, and at this time point monocytes presented the highest values in athletes (Figure 2). This value was also significantly different ($P < .001$) from the control group.

Eosinophils

There was no significant interaction of group and time ($F_{2,32} = 0.04$, $P > .05$). In athletes, eosinophils decreased from $0.106 \pm 0.035 \times 10^9/L$ preexercise to $0.082 \pm 0.037 \times 10^9/L$ postexercise, but this change was not significant ($P > .05$).

Basophils

There was no significant interaction of group and time in basophils ($F_{2,32} = 2.55$, $P > .05$), and no differences were observed in either group at any time point or between athletes and the control group.

---

**Figure 2** — Leukocyte subpopulations (mean ± SD) in athletes and controls at different time periods (pre: 8 AM; post: 12:30 PM; 4 h: 4:30 PM) after the athletes underwent a 2-h training session. **Significantly different from athletes ($P < .01$). ***Significantly different from athletes ($P < .001$). ††Significantly different from preexercise ($P < .01$). †††Significantly different from preexercise ($P < .001$).
The significant interaction of group and time (F\(_{2,32} = 9.17, P < .001\)) revealed a different pattern of cortisol concentration between groups (Figure 3). Although cortisol in athletes reached the highest concentration immediately after exercise, this increase (7.9%) was not significant (P > .05) compared with preexercise values. At 4 hours cortisol concentration decreased significantly (P < .01) in athletes (52.7% relative to preexercise levels). The pattern of cortisol changes in the control group followed the circadian rhythm, revealed a significant decrease (P < .01) at the second time point (post), and remained reduced at the 4-hour time point and significantly different from the baseline values (P < .001). In addition, the concentration of cortisol after exercise was significantly higher (P < .001) in athletes than in the control group, but at 4 hours these differences had disappeared (P > .05).

**Discussion**

In the current study we examined the acute changes in leukocyte number and cortisol after a single bout of soccer training in female elite players and found that after the end of the training session, total white blood cell count increased significantly and was 70% higher than the preexercise values. These results are in line with the
findings of other investigators who have observed leukocytosis during and after the end of exercise.\textsuperscript{13,18} White blood cell count might remain elevated far longer than the duration of the exercise itself,\textsuperscript{19,20} and this was also confirmed by the results of our study because 4 hours later leukocytes were 78\% higher than preexercise values. In general, the magnitude and permanence of leukocytosis appears to be directly related to exercise intensity and duration.\textsuperscript{4} Results of several studies indicate that exercise duration seems to be the most important factor.\textsuperscript{5,21} In the current study, the duration of the exercise session was 2 hours, and it was performed at a moderate intensity (75\% HRmax). Comparable changes have been observed in studies with similar intensity and duration in the concentration of total white blood cells ~3.5 hours after exercise cessation.\textsuperscript{19,21,22}

With reference to leukocyte subsets, neutrophils were 142\% higher postexercise and 152\% higher after 4 hours recovery than preexercise values. This result affected the changes in total white blood cells, because the neutrophils account for up to 40\% to 50\% of the immune cells in circulation.\textsuperscript{23} Bishop et al\textsuperscript{13} reported a similar increase in neutrophils (approximately 139\%) after 90 minutes of a soccer-specific exercise protocol at an intensity of 84\% of age-predicted maximum heart rate in university team soccer players. Malm et al\textsuperscript{12} found a smaller increase in neutrophils after 2 consecutive soccer games separated by 20 hours. That study, however, did not take any measurements after the first game or immediately before the second game, making it difficult to assess the extent of any “carryover” from the first game. Furthermore, the games were held at different times of day, and thus it could be argued that diurnal changes might have had some influence on the results.\textsuperscript{24} In our study, the duration of the exercise session was 120 minutes, and perhaps this caused a considerable rise in neutrophils. This rise is connected with the increase in plasma cortisol concentration,\textsuperscript{5,25} because it has been shown that corticosteroids cause neutrophilia.\textsuperscript{5} According to McCarthy and Dale,\textsuperscript{5} cortisol concentration increases when the intensity of exercise is higher than about 60\% of VO\textsubscript{2}max. In this case, neutrophils are activated immediately because they constitute an essential part of innate immunity and are the first cells to localize to sites of injury and inflammation. It is thought that they play an important role in phagocytosis and degradation of damaged tissue, especially in skeletal-muscle damage during intensive exercise, releasing chemical substances that are involved in inflammation.\textsuperscript{4}

In contrast to neutrophils, lymphocytes did not present remarkable changes in our study. There was a small decline (6.3\%) after the cessation of the training session, but 4 hours later, they returned to preexercise values. Similar findings have been reported after a number of different exercise protocols with moderate intensity,\textsuperscript{13,18} when the lymphocyte count remained almost unchanged after the exercise. The limited possibility of direct response of lymphocytes during exercise is that this subclass of leukocytes is responsible for the acquired immune response.\textsuperscript{26} A significant decrease in lymphocytes that leads to suppression of immune function has been noted after long-duration (>3 hours) and high-intensity exercise; a decrease in lymphocytes was not observed in the current study. Furthermore, Benschop et al\textsuperscript{27} proposed that catecholamines predominantly affect natural-killer-cell and granulocyte circulation, whereas T- and B-cell numbers (which constitute the major subsets of lymphocytes) remain relatively unaffected. The changes in lymphocyte circulation seem to be mainly mediated via activation of β\textsubscript{2}-adrenoceptors, whereas granulocyte increases involve α-adrenoceptor stimulation. Our data,
however, cannot provide any conclusions about these mechanisms because we did not measure catecholamines.

After the single bout of soccer training, monocytes increased by 27% postexercise and 41% at 4 hours after exercise from preexercise resting levels. In agreement with our findings, most studies with moderate-intensity exercise indicate a modest increase in the total number of circulating monocytes. It is worth mentioning that 4 hours after exercise, monocytes were significantly higher in our soccer players than in the control group. This pattern probably results from the actions of monocytes during and after exercise, because they have been shown to localize and infiltrate the damaged skeletal muscle. Recruitment of monocytes into the circulation is immediate because they are involved in innate immune function.

Basophils remained unchanged after the exercise session in the current study. Data show that moderate- (50% VO\textsubscript{2} max) and high-intensity (80% VO\textsubscript{2} max) exercise do not induce considerable changes in circulating basophils. Few data, however, are available concerning the effects of exercise on basophils. Moreover, the total number of cells counted is so small that it is difficult to draw valid conclusions. Furthermore, the eosinophils, which constitute a small percentage of circulating leukocytes, follow similar patterns. In our study, we observed a small decline after the exercise session and 4 hours later, even though these changes were not significant. Concurring with the current study, another study showed a reduction in these cells after prolonged and short-term vigorous exercise.

Many of the changes in innate immunity we have outlined correlate with stress hormones, particularly with cortisol. Cortisol has generally been thought of as an immunosuppressive and anti-inflammatory agent. The single bout of soccer training in our study did not result in a significant increase (7.9%) in cortisol concentration. There was a considerable difference, however, between athletes and the control group at the same time point (12:30 PM). This is probably because of diurnal variation of cortisol, which peaks early and then declines throughout the morning. Thus, the results of the current study reveal that cortisol remained elevated immediately after the exercise session in the athlete group and was reduced 4 hours later, reaching levels similar to those of the control group. Consistent with the current study, Hoffman et al found that cortisol concentrations were influenced by diurnal variation before and after a competitive football game. In addition, there is evidence that high-intensity exercise (>60% VO\textsubscript{2} max) results in a significant elevation in cortisol concentrations. Generally, the mean relative work rate in soccer has been reported to be around 70% of VO\textsubscript{2} max. If the bout is sufficiently prolonged, however, even relatively moderate intensities can elicit increases in cortisol because it is released to increase gluconeogenesis and maintain blood glucose concentration. Our results support this aspect, because the exercise intensity was moderate (75% HRmax) and the duration was prolonged (2 hours).

**Practical Applications**

The current study provides a detailed description of changes in leukocyte distribution after exercise in elite female soccer players. Knowledge in this area could optimize the development of training programs, because soccer frequently involves more than 1 exercise session per day and many in-season contests. Our data show that after a single bout of soccer training in elite female athletes, circulating leukocyte numbers
increased markedly and remained elevated 4 hours after the cessation of exercise. This leukocytosis is probably produced by the release of neutrophils from the bone marrow. This fact potentially reflects the process of phagocytosis and degradation of skeletal muscle damaged during exercise. It is probable that a time period of more than 4 hours, until the next exercise session, would normalize cellular components of innate immunity. It is also currently unclear whether these changes are sufficient to have major clinical implications. We recognize, however, that further research is required to determine additional cellular and humoral immune components after a single bout of soccer training in elite female athletes.

**Conclusions**

A single bout of soccer training induced significant leukocytosis in female players primarily because of neutrophilia, which was still evident after 4 hours of recovery. Furthermore, cortisol concentration was significantly higher in athletes than in nonathlete control subjects after the training session. Coaches and conditioners should provide more than 4 hours between exercise sessions. On the other hand, lymphocyte count was not affected significantly by a 2-hour training session at an intensity of 75% of HRmax. The latter observation suggests that in elite female players this specific type of exercise does not induce a significant decrease in lymphocyte count and probably does not reduce the ability to produce an effective response against a possible viral infection.

**Acknowledgments**

The project was cofunded by the European Community Fund (75%) and National Funds (25%) EPEAEK II-HRAKLEITOS.

**References**


