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Changes in Several Neutrophil Functions in Basketball Players Before, During and After the Sports Season

G. Benoni [1], *P. Bellavite* [2], *A. Adami* [1], *S. Chirumbolo* [2], *G. Lippi* [2], *G. Brocco* [2], *G. M. Giulini*, *L. Cuzzolin* [1]

[1] Institute of Pharmacology and [2] Institute of Chemistry and Clinical Microscopy, University of Verona, Verona, Italy

Abstract

G. Benoni, P. Bellavite, A. Adami, S. Chirumbolo, G. Lippi, G. Brocco, G. M. Giulini and L. Cuzzolin, Changes in Several Neutrophil Functions in Basketball Players Before, During and After the Sports Season, Int. J. Sports Med., Vol. 16, No. 1, pp. 34–37, 1995.

Accepted after revision: May 25, 1994

Neutrophils play an important role in the immune system, forming the "first line of defence" against invading microorganisms and there are few data available concerning neutrophil functions in relation to exercise. We investigated in 7 basketball professional players possible changes before, during and after the sports season, in some haematological parameters and in several aspects of the phagocytic process of neutrophils, such as adhesion, superoxide anion release and bactericidal activity. Training and competitions produced a significant rise in the number of total leukocytes and differential counts, but the values returned to the pre-start levels 3 weeks after the end of the championship. The bactericidal activity and the superoxide anion released were significantly greater during the sports season, while the percentage of cellular adhesion significantly decreased during the championship; after the sports season the values returned to the control levels. As in the literature data concerning neutrophil functions in relation to exercise are non-convergent, it is important in our opinion, to understand whether the alterations induced by exercise can persist after repeated stimuli.

Key words

Basketball players, neutrophil functions, immunological parameters

Introduction

The influence of exercise on the immune function is an important community health issue that encompasses a wide range of activities, from recreational jogging to the performance of elite athletes undertaking training programs. The optimal level of exercise that enhances, but does not suppress the immune function is unknown (15). Some authors observed that runners and joggers claim a state of physical well-being in which they are less susceptible to infections (18), while it is a common observation in sports medicine that highly trained athletes are more subjected to infectious diseases (6,23), probably due to the greater physical and psychological stress experienced (5).

Neutrophil granulocytes play an important role in the immune system, forming the "first line of defence" against invading microorganisms (20) and there are few data available concerning neutrophil functions in relation to exercise. Moreover many of the observed changes about immune parameters, like leukocytosis (7,12) and increase in natural killer cells (11) appear to be transient, returning to normal within hours after the exercise stimulus.

The aim of this work was to investigate in professional basketball players possible changes, before, during and after the sports season, in some haematological parameters and in several important aspects of the phagocytic process of neutrophils, such as adhesion, superoxide anion release and bactericidal activity.

Materials and Methods

Subjects

The group of elite sportsmen participating in the study consisted of 7 athletes between 20 and 30 years of age from the "GLAXO" professional team. Only those basketball players who had had no symptoms of illness or infection in the previous 2 months were selected for the study. None had taken drugs or any other medication in the 2 months prior to the study period. The investigation was performed before, during the basketball training and competition program (in the middle of the season) and 3 weeks after the end of the championship. The training program followed by these athletes is the common program for an elite basketball team, based normally on "hollow sprints", interval training, sprint training, weight exercises and technical exercises lasting some 3 h/day; the competition program consisted of one game a week.

Protocol

Peripheral venous blood samples were drawn by antecubital venepuncture and collected into EDTA-containing tubes. The blood was taken at 8 a.m., the day after the competition, before, after 4 months from the beginning and 3 weeks after the end of the sports season. The samples were immediately sent to our laboratory for the assays.

Int. J. Sports Med. 16 (1995) 34–37
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Analytical methods

The determination of red blood cell count (RBC) was made with a Technicon H6000 automatic analyser; total leukocytes and differential counts were estimated by standard procedures (Coulter Counter T 660).

Separation of neutrophils

The neutrophils were prepared from ethylene-EDTA anticoagulated blood which was fractionated by different centrifugations over Percoll gradients. The cells (>98% of purified neutrophils) were finally resuspended in Hank's balanced salt solution (HBSS) supplemented with 0.2% human serum albumin, 5 mM glucose, 0.5 mM $CaCl_2$ and 1 mM $MgSO_4$ (HCGMA) and immediately used at the concentration of 4×10^6 neutrophils/ml for superoxide anion assay and 5×10^6 cells/ml for bactericidal activity. Cell viability was checked by the trypan blue exclusion test and in all cases was greater than 95%.

Bactericidal assay

A wild strain of *Staph. aureus,* grown in Brain Heart Infusion for 16 h at 37 °C, was used in the experiment. The suspension of bacteria was then centrifuged at 3000 rpm for 10 min and, discarding the supernatant, resuspended in HCGMA. The concentration of bacteria in the suspension was adjusted to obtain an O.D. (Optical Density) against a buffer blank at 620 nm approximately to 0.200, corresponding to about 5.12×10^8 bacteria/ml. Scalar dilutions 1:1 from the starting suspension (named D_0) were prepared until D_7. 25 µl of these dilutions were delivered in triplicate into the wells of a 96-well sterile microplate with flat bottom (Limbro-type, FLow). Then the wells were supplemented with 25 µl of 16% pooled human sera (diluted in HBSS) and, after 10 min, with 50 µl of the neutrophil suspensions or 50 µl of HCGMA in control samples. The incubation was carried out for 60 min at 37 °C in a humidified thermostat, then after the killing of the cells the plates were prepared using Brain Heart Infusion as medium. Bacterial growth was followed at interval times (every hour from 0 to 5 hours) by reading the O.D. at 620 nm with a microplate reader. Bactericidal capacity of human neutrophils was evaluated by comparing the bacterial growth of samples incubated with neutrophils to the bacterial growth in the absence of cells.

Superoxide anion assay

The superoxide anion was measured by the superoxide dismutase (SOD)-inhibitable reduction of ferricytochrome c modified for microplate assay (1). As preliminary experiments showed that neutrophils spontaneously adhered to the bottom of the wells and produced considerable amounts of O_2^-, nonspecific activation was totally abolished by coating the microplate wells with 100 µl of fetal bovine serum (FBS) for at least 2 h at room temperature: immediately before use, the plates were washed three times with phosphate saline buffer (PBS) using an automatic plate washer (SLT Lab. Instr.). Operatively, the wells were supplemented with 25 µl of fMLP 10^{-7} M as stimulant, 25 µl of cytochrome c as the probe for the detection of O_2^-, and finally 50 µl of the neutrophil suspension prewarmed at 37 °C for 10 min. As controls, 25 µl of HCGMA, 25 µl of cytochrome c and 50 µl of the cells without the stimulant were added in the wells. The plates were then brought to 37 °C in a humidified incubator throughout the experiment. When indicated (at 5, 10, 20, 30 and 40 min) the plates were rapidly transferred into a microplate reader (READER 400, SLT Labs Instr.) and the reduction of cytochrome c was measured at 550 nm using 540 nm as a reference wavelength to avoid interference due to light scattering. In all procedures, care was taken to avoid cooling of the plate when it was taken from the incubator during handling and taking readings. To obtain the nmol of superoxide anion produced, the O.D. of the sample was divided by the O.D. of the ferricytochrome c reduced (1 nmol = 0.04 O.D.). All the tests were made in triplicate.

Adhesion

The endothelium adherence capacity was measured immediately after the detection of the superoxide anion according to the method of Bellavite et al. (1). The microplate was subjected to two washing cycles with PBS at room temperature and the cellular adhesion was evaluated by measuring the membrane enzyme acid phosphatase. 75 µl of an acetate buffer 0.15 M, pH 5.3, containing 0.2% Triton X-100 were plated: after 5 min at room temperature 75 µl of 0.15 M acetate buffer containing the substrate (10 mM p-nitrophenyl-phosphate) were added. After incubation at room temperature for 20 min, the reaction was stopped by adding 100 µl of NaOH 2 N. The p-nitrophenol production was measured spectrophotometrically at 405 nm. The percentage of cellular adhesion was calculated on a standard curve obtained with known numbers of neutrophils. The test was made in triplicate.

Statistical analysis

All the values were expressed as the mean ± S.D. The statistical evaluation of the data was carried out by applying the ANOVA test.

Results

The total number of red blood cells (RBC) and white blood cells (WBC) as well as the number of neutrophils, lymphocytes, monocytes and eosinophils in the 7 athletes studied before, during and after the sports season are shown in Table **1**. Training and competitions produced a significant rise in the number of total leukocytes and differential counts, but the values returned to the pre-start levels 3 weeks after the end of the championship.

The results of the bactericidal assay are shown in Fig. **1**. We evaluated the bacterial dilution D_3 since the results

Table 1 Haematological parameters in our 7 athletes before, during and after the sports season. The values are expressed as mean ± SD.

	Before	During	After
RBC (10^{-3}/µl)	5.25 ± 0.56	5.25 ± 0.84	5.16 ± 0.34
WBC (10^{-3}/µl)	4.51 ± 0.81	6.83 ± 0.79**	4.44 ± 1.05
Neutrophils (10^{-3}/µl)	2.58 ± 0.62	3.58 ± 0.80*	3.04 ± 0.70
Lymphocytes (10^{-3}/µl)	1.54 ± 0.34	2.48 ± 0.72*	1.58 ± 0.40
Monocytes (10^{-3}/µl)	0.27 ± 0.09	0.43 ± 0.08**	0.33 ± 0.25
Eosinophils (10^{-3}/µl)	0.08 ± 0.02	0.14 ± 0.05*	0.08 ± 0.03

*$p < 0.05$; **$p < 0.005$

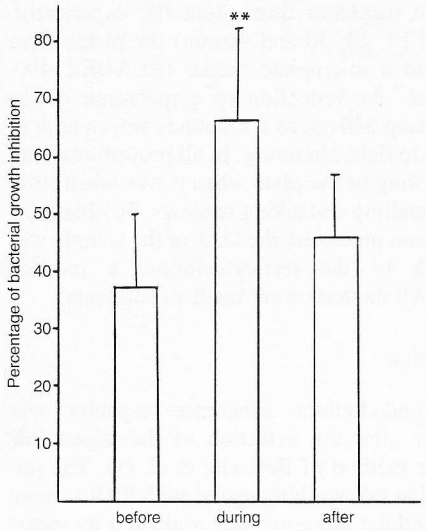

Fig. 1 Bactericidal activity, detected at 5 h, of neutrophils obtained from our 7 athletes. The values are expressed as mean ± SD.

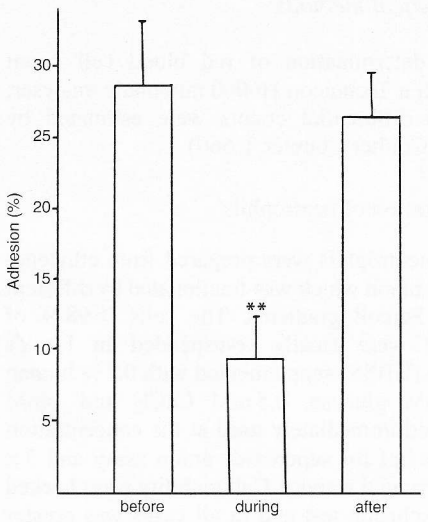

Fig. 3 The percentage of cellular adhesion with fMLP 10^{-7} M as stimulant in our 7 athletes. The values are expressed as mean ± SE.

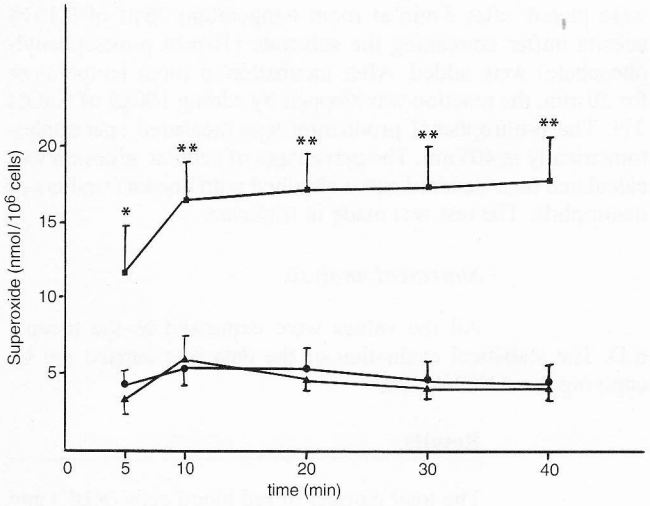

Fig. 2 Superoxide anion production with fMLP 10^{-7} M as stimulant before (●), during (■) and after (▲) the sports season in our 7 athletes. The values are expressed as mean ± SE.

for the ratio cells/bacteria were better in this and in previous experiments. The bactericidal activity, expressed as percentage of growth inhibition, was significantly greater ($p < 0.005$) during the sports season, indicating a strong influence of training on this parameter.

The superoxide anion released by neutrophils isolated from our athletes before, during and after the sports season is given in Fig. **2**. The values, significantly increased during the sports season, returned to the control values 3 weeks after the end of the championship.

The percentage of cellular adhesion before, during and after the sports season is shown in Fig. **3**. We observed a higher percentage of adhesion in our athletes before and after the sports season, while this parameter of leukocyte function significantly decreased ($p < 0.001$) during the championship.

Discussion

There are few data available concerning neutrophil functions in relation to chronic exercise, despite the ability of neutrophil granulocytes to protect the body against infections (8). Waller (23) has indicated that better-designed studies are needed to determine whether the effects of exercise enhance or suppress the immune response.

Recent investigations in top athletes indicate that intense training during a competitive period may suppress the immune system and increase susceptibility to infections (9, 18). However, most of these studies have evaluated athletes immediately after their participation in competitive events involving maximal exertion and stress, thus it is impossible to know whether the observed alterations in immune functions are due to the outpouring of hormones that occurs during intense stress induced by exercise, to the exercise itself or to both (2). Moreover, many of the observed changes appear to be transient and return to normal within a short time (14). Finally, the duration of the training period and the sport being studied are important parameters that might explain the conflicting results observed in the literature.

For all these reasons, we also studied the phagocytic process of neutrophils from our highly trained athletes at rest (3 weeks after the end of the championship) and not immediately after the sports season.

With regard to the number of immune cells, our results show a significant increase in the WBC total number and in the differential counts during the sports season, compared to other published data that indicate the appearance of leukocytosis related to physical exercise (3, 12, 17). Nevertheless, three weeks after the end of the championship the values returned to the pre-start levels in agreement with other studies (23).

In recent years, some authors found that the phagocytic function is stimulated after acute exhausting exercise both in peritoneal macrophages (4, 15) and in blood neutrophils (19), but the potential of the neutrophil population to kill foreign pathogens is depressed chronically by an intensive training (22).

Adhesion is the early event in the neutrophil participation in the host defence mechanisms. The adherence capacity of neutrophils significantly decreased during the sports season in our subjects, while other authors (21) have indicated an increase in this neutrophil function over the duration of the training program in football players. It is possible that the influence of exercise on this leukocyte function depends on the sport being studied: the effect of basketball could be different from that of football, since the duration and programs of training followed are different. Moreover the exercise, which is a form of stress (2) increases the plasma levels of many hormones, including glucocorticoids and some authors (13) have reported that the adherence capacity is decreased by glucocorticoids.

Concerning the next stage of the phagocytic process we found an increase of bactericidal activity in the middle of the competitive season, which is in agreement with Ortega et al. (16) and Lewicki et al. (10), although these authors measured this process by other techniques (microbicide index against *C. albicans* after 60 min of incubation) and they did not give any indication about the values at rest, comparing trained to untrained subjects.

Finally, we observed a significant increase in superoxide anion production during the sports season. There is a number of possible explanations for the observed alterations in this parameter: exercise-induced enhancement of neutrophil oxygenation activity could be due to cytokines released into the circulation during exercise or, more indirectly, to the selective release from isolated vascular pools of a subpopulation of neutrophils with intrinsically higher activity (22).

Since the few data available in the literature concerning neutrophil functions in relation to exercise are non-convergent, as a consequence of the different methods used, of the characteristics of the exercise (duration and workload) and of the type of the athletes tested, in our opinion, this argument requires to be further elucidated by investigating and comparing same cohorts of athletes before, during and after a period of intense training.

It is important to understand if the alterations in the leukocyte functions induced by exercise, even if they are transient, can persist after repeated stimuli.

Acknowledgements

This work was supported by grants from Consorzio per lo Sviluppo degli Studi Universitari, Verona, Italy.

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Dr. Giuseppina Benoni

Institute of Pharmacology
University of Verona
Policlinico Borgo Roma
I-37134 Verona
Italy