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## Anemia and immunological profile in young female Cameroonian professional handball players

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### Abstract

The physiological demands of modern sports competition involve biological monitoring of athletes which unfortunately is scarce in African countries. The aim of this study was to study the variations in hematological and immunological parameters after a two-week training session in Cameroonian handball players. 29 handball players aged  $25 \pm 2$  years (height:  $1.65 \pm 0.06$  m; weight:  $64.47 \pm 6.21$  Kg; BMI:  $23.62 \pm 2.46$  Kg/m<sup>2</sup>) followed a specific training program two weeks with blood samples taken at the start and end of the program to determine the variation in their blood count. More than half (15) of the participants had hemoglobin levels below normative values. A significant increase in the amount of leukocytes in the anemic group (10.85%,  $p < 0.05$ ) and a non-significant decrease in the "normal" group (2.46%,  $p > 0.05$ ) were observed. The individual analysis of the different types of leukocytes shows contrasting variations between the 2 groups of subjects. Basophils and eosinophils decreased in anemic sportswomen ( $p > 0.05$ ) and increased in "healthy" sportswomen ( $p > 0.05$ ). Lymphocytes and monocytes increased in anemic sportswomen (17.71% and 1.05% respectively) and decreased in "healthy" sportswomen (4.75% and 12.55% respectively). In anemic subjects, the increase in lymphocytes was very significant ( $p < 0.05$ ) compared to "healthy" subjects. The leukocyte disturbances in response to the training program elicited in anemic sportswomen, require regular monitoring of biological markers.

**Keywords:** Female athletes, blood count, aerobic exercise, Cameroon

### Introduction

Optimizing physical performance in athletes today requires regular biological monitoring. Regular biological monitoring helps to identify the thresholds for hematological, physiological or immunological parameters which are compatible with good practice in the chosen sport discipline [1]. Thus, parameters such as maximum oxygen consumption (VO<sub>2</sub>max), maximum aerobic speed (MAS), ventilatory and lactic thresholds, perception of exertion, hemoglobin concentration or the profile of certain leukocyte elements are important determinants to athletic performance [2, 3, 4]. In the scientific literature, there are many studies which provide information on the normative values of the above-mentioned parameters and therefore require corrections of these when athletes are subjected to exhausting training or which promote the alteration of these biological indicators [5, 6, 7]. This aspect is particularly frequent in female athletes in view of the particular female physiology that sets in from the age of reproduction [8, 9, 10]. Thus, regular iron supplements can be observed in some sportswomen engaged in competition who have a deficiency in this ion due to numerous physiological mechanisms relating thereto (gastrointestinal, genitourinary and menstrual hemorrhage, intravascular hemolysis, iron deficiencies by sweating or nutritional iron deficits) [11, 12, 13]. On the immune system, acute prolonged periods of exercise and periods of intensive training are associated with a temporary increased risk of infection. In general, the effects of exercise on the immune system are highly dependent on the mode and intensity of exercise or training. More attention should therefore be paid to female athletes especially on the eve of competitive periods. Much work in Caucasian female athletes has shown that aerobic-type exercise induces inflammation and hormonal activity which can interfere with iron metabolism [14, 15]. This suggests a medico-sports approach through individualized medical monitoring to maintain, preserve or improve

the state of health of athletes in order to seek performance in optimal conditions. This is why markers such as hemoglobin level and serum ferritin are generally used in athletes in endurance disciplines as a means of screening their blood status [7]. Thus, in some athletes during a competitive period, we will proceed to a rigorous iron supplementation. However, these iron supplements are not accompanied by a change in immunological parameters in these athletes. The issue of biological monitoring of athletes is less discussed in African circles. However, these athletes are subject to the same physiological demands of athletic competition. In Cameroon, a study by Amar Moor *et al.* [16] revealed that the blood profile of certain biomarkers such as hematocrit and hemoglobin levels were decreased over different periods in elite male athletes. This observation is consistent with the same conclusions encountered in Caucasians and appears to be sufficient justification for the monitoring or early detection of these markers, especially in female athletes in whom the situation would be exacerbated. Knowledge of these data would also be an asset for these athletes who participate in African and world competitions with more or less satisfactory results. In a context marked by a scarcity of studies on the biological monitoring of female athletes, the present study therefore aimed to study the variations in hematological and immunological parameters after a two-week training session in Cameroonian handball players.

## Materials and Methods

### Participants

Twenty-nine (29) participants belonging to the handball-ladies team of the National Institute of Youth and Sports (INJS), took part in this study. The INJS women's handball team competes in the national civil handball championship with players who regularly participate in the training sessions. Each player reported having a 28-day menstrual cycle. The ethical considerations have been validated by obtaining an ethical clearance issued by the regional ethics committee for research in human health for the central region under number 2018/0592/ CEIRSH/ ESS/MSP. The study was carried out in strict compliance with the fundamental principles of medical research according to Helsinki's Declaration.

### Procedure

The experimental protocol took place at the INJS on the one hand, for the taking of anthropometric parameters and at the Human Biology laboratory of the Institute for Medical Research and Studies of Medical Plants (IMPM) of the Ministry of Research, Scientist and Innovation from Cameroon, for taking hematological parameters on the other hand.

### Anthropometric parameters

For the body weight measurement, the player climbed the scale in light clothing, barefoot, bust straight, gaze horizontal. The reading was done directly on the dial. For the standing height measurement, the subject was barefoot and together, gaze horizontal. The experimenter slid the square of the measuring board and placed it above the subject's head. The reading was made on the upright of the measuring board at the level of the lower base of the square. NB: the body mass index (BMI) was calculated for each participant according to Quetelet's formula [17] by dividing the body weight (kg) of each subject by the square of his standing height (m).

### Blood count

The blood count was determined using a Humacount brand

multiparameter machine (Westbaden, Germany). The samples were taken by a nurse and in the presence of a referring biologist. The sportswomen were on an empty stomach and the assessment was carried out in the morning, at 8 o'clock. No training had been offered to the players the day before the tests. The samples were taken after checking the identity of the athletes and the labeling of the tubes was done at the time of the sample. The leukocyte formula (polymorphonuclear neutrophils, eosinophils, polymorphonuclear cells, lymphocytes, monocytes), hematocrit, hemoglobin level, mean blood volume of the participants were thus measured. For each subject, two samples were taken: one before the training program and the other two weeks after said program. During this time, no handball player was on contraception or in her menstrual period.

### Training Program

The specific training program administered to the athletes lasted two (02) weeks. The training sessions were daily and lasted 1h30mm. The specific training program consisted of performing exercises whose intensity and volume were close to those of competitive matches. This program was as follows: - intermittent races (30-30 and 15-15) that is to say 30 seconds of running at maximum speed followed by 30 seconds of passive recovery (walking); 15 seconds of running at maximum speed followed by 15 seconds of passive recovery (walking); - outnumbered ball games; - plyometric exercises; - multi bonds; - counter-attacks (at 2, at 3); - duels in attacking situations (one against one, three against two...); - duels in a defensive situation; - specific exercises on goalkeeper parades; - relationship games for 2 or 3 in offensive and defensive situations; - outnumbered games all over the field

### Statistical analysis

Data were presented in tabular form with means±standard deviation. Analysis was performed with graph pad 5.03 software. The paired T test was used to compare the start and end means for each parameter. The results of different classes of leukocytes were expressed in microliters and as a percentage. The significance threshold was set at 5% ( $p<0.05$ ). The rate of change of the leukocyte parameters was calculated according to the following formula:  $T (\%) = \frac{Cf - Ci}{Ci} \times 100$  with T: rate of change of the parameter between the first sample and the second sample, in%; Cf: Concentration of the final value of the parameter; Ci: Concentration of the initial value of the parameter.

### Results

Table 1 shows the mean values of the anthropometric parameters in the overall sample (n = 29); the subjects appear to be generally normal-weight.

**Table 1:** Anthropometric characteristics of participants

Parameters	Mean±Standard Deviation (n=29)
Age (years)	24.75±1.75
Weight (kg)	64.47±6.21
Height (cm)	1.65±0.06
BMI (kg/m <sup>2</sup> )	23.62±2.46

n: sample size; BMI: body mass index

Table 2 shows that red blood cells, hemoglobin and hematocrit underwent insignificant decreases ( $p,00.05$ ) after 2 weeks of training. A non-significant increase ( $p>0.05$ ) in white blood cells was observed. Lymphocytes significantly

increased ( $p<0.05$ ). The other parameters of the leukocyte formula such as monocytes and eosinophils decreased (non-significant difference, ( $p>0.05$ ).

**Table 2:** Variations in the amounts of leukocyte types before and after the training program

Parameters (n=29)	Mean±Standard Deviation		% variation	P-value
	Before	After		
Hb (g/dL)	11.77±1.21	11.66±1.16	-0.93%	0.53
Hct (%)	34.72±2.9	34.57±3.11	-0.43%	0.90
RBC (x10 <sup>6</sup> /μL)	4.37±0.35	4.28±0.44	-2.5%	0.20
WBC (x10 <sup>3</sup> /μL)	4.15±0.96	4.32±0.90	+4.09%	0.19
MCV (fl)	79.71±6.65	80.33±7.39	+0.77%	0.01
MCHC (g/dL)	33.77±0.22	33.66±0.13	-0.32%	0.56
MCHL (pg)	27.25±3.49	27.09±2.79	-0.58%	0.56
PLT (x10 <sup>3</sup> /μL)	210.13±42.09	224.48±50.84	+6.82%	0.01
MPV (fl)	9.45±1.08	9.52±1.01	+1.55%	0.53
Lymph (nb/μL)	1805.68±503.69	1982.31±470.43	+9.78%	0.01
Mono (nb/μL)	289.07±116.50	255.92±114.77	-11.76%	0.14
Neutro (nb/μL)	1885.37±554.78	1975.1±589.84	+4.75%	0.44
Eosino (nb/μL)	135.2±82.64	99.48±65.98	-26.15%	0.10
Baso (nb/μL)	22±10.32	25±11.78	+13.63%	0.34

Analysis of hemoglobin levels revealed anemic and normal subjects. The tables below present a comparison of some hematological parameters between these 2 groups.

**Table 3:** Variations in the amounts of leukocyte types before and after the training program in the group of anemic subjects

Anemic subjects (n=15)	Mean±Standard Deviation		% variation	P-value
	Before	After		
Hb (g/dL)	10.77±0.62	11.14±0.73	+10.14%	0.53
WBC (x10 <sup>3</sup> /μL)	3.87±0.65	4.29±0.85	+10.85%	0.03
Lymph (nb/μL)	1654.00±406.00	1947.00±372.70	+17.71%	0.001
Mono (nb/μL)	264.80±79.30	267.60±90.79	+1.05%	0.92
Neutro (nb/μL)	1807.00±448.80	2009.00±580.80	+11.17%	0.27
Eosino (nb/μL)	156.20±99.57	105.40±69.30	-32.52%	0.17
Baso (nb/μL)	39±21.28	25±5.00	-35.89%	0.45

n: sample size; Hb: hemoglobin level; WBC: white blood cells; Lymph: lymphocytes; Mono: monocytes; Neutro: neutrophils; Eosino: eosinophils; Baso: basophils

**Table 4:** Variations in the amounts of leukocyte types before and after the training program in the group of subjects with normal blood counts

Normal subjects (n=14)	Mean±Standard Deviation		% variation	P-value
	Before	After		
Hb (g/dL)	12.86±0.59	12.22±1.30	-11.21%	0.02
WBC (x10 <sup>3</sup> /μL)	4.46±1.16	4.35±0.99	-2.46%	0.49
Lymph (nb/μL)	1956.00±394.00	1863.00±380.90	-4.75%	0.33
Mono (nb/μL)	374.30±154.30	327.30±136.00	-15.55%	0.17
Neutro (nb/μL)	1780.00±484.60	1804.00±537.50	+1.34%	0.87
Eosino (nb/μL)	102.50±45.27	103.30±71.13	+0.78%	0.96
Baso (nb/μL)	22.22±10.93	24.44±12.36	+9.99%	0.51

n: sample size; Hb: hemoglobin level; WBC: white blood cells; Lymph: lymphocytes; Mono: monocytes; Neutro: neutrophils; Eosino: eosinophils; Baso: basophils

It appears from Table 3 that white blood cells increased significantly ( $p<0.05$ ) in anemic subjects after 2 weeks of training. This increase is mainly dependent on that of lymphocytes whose percentage increase was of the order of 17.71%. In contrast, in normal subjects (Table 4), white blood cells did not increase significantly. A decrease (non-

significant difference,  $p>0.05$ ) in hemoglobin was observed in normal subjects after 2 weeks of training. In contrast, the anemic subjects showed an increase in their hemoglobin level (non-significant difference,  $p>0.05$ ). In the other parameters of the leukocyte formula, several contrasting profiles are observed between anemic subjects and normal subjects; while eosinophils and basophils remained slightly stable in normal subjects, they were reduced in anemic subjects. However, lymphocytes and monocytes increased in anemic subjects while the same parameters rather decreased in normal subjects.

## Discussion

The general objective of the present study was to study the variations in hematological and immunological parameters after a two-week training session in Cameroonian handball players. The body mass index values indicate that the participants were of normal build and regularly engaged in physical activities and sports. Analysis of hemoglobin levels revealed an almost similar proportion of anemic and non-anemic athletes. This result confirms the common observations encountered in female athletes on their blood status. Indeed, many studies argue in favor of regular monitoring of athletes during competitive periods, particularly their iron status [18, 19]; Competition and training have been shown to be associated with iron deficiency in these athletes. The resting hemoglobin values found (10.77±0.62 g / dL) in our study in anemic athletes indicate iron deficiency anemia according to the classification of Peeling *et al.* [7]. However, the slight increase observed after 2 weeks seems quite paradoxical and is linked to the intensity of the training. On the other hand, the decrease in hemoglobin level observed in non-anemic athletes is in agreement with the scientific literature and thus reflects a deleterious effect of aerobic exercise on the amount of iron in the body [20, 21]. The hemoglobin level values in handball players with a normal blood count are similar to those in professional footballers, with a hemoglobin status dependent on the energy mobilized depending on the sport discipline [21]. Athletes in mixed energy sports (anaerobic and aerobic) generally have low values compared to athletes in aerobic or anaerobic energy disciplines [21]. Dividing the athletes into 2 groups (anemic athletes and non-anemic athletes) led to different observations of their leukocyte profile after 2 weeks of training. The comparison of the variations of the white blood cells during the experiment, showed that the group of anemic subjects presented at the beginning, a slight leukopenia with an average value of 3.87±0.65 x 10<sup>3</sup> / μL, slightly lower than the usual reference range (4.00 x 10<sup>3</sup> to 10.00 x 10<sup>3</sup> / μL of blood), before undergoing a significant increase of + 10.85%, after 2 weeks of training; These results are similar to those of Pedersen and Bruunsgaard [22]. Said *et al.* [23] had shown that the number of leukocytes increases significantly, both in endurance and resistance athletes after maximum physical exercise at increasing load (Vameval test) or at constant load (time limit test), sustained until exhaustion. In subjects with normal blood counts, our results showed a non-significant decrease in the number of lymphocytes of -2.46%; Indeed, Gleeson [24] reports that very prolonged exercise sessions and periods of intensive training or competition cause a decrease in the function of white blood cells. In the work of Walsh *et al.* [25], prolonged sessions of strenuous exercise have been shown to cause a temporary decline in white blood cell function and that these changes create a breeding ground for a decline in the protection during which viruses and bacteria

can break down. implant, also increasing the risk of developing an infection. During the 2-week training program, none of the participants fell ill despite this drop. The decline in immune defenses remains transient and is thought to be greatest during the period of 3 to 72 hours following the end of exercise<sup>[26]</sup>; our subjects had their second blood count test 24 hours after the training program. Lewicki *et al.*<sup>[27]</sup> also reported the decrease in the immune response during physical activity. The participants were subjected to intense drills, identical to those of a high-level handball meet, one hour and 30 minute practice sessions. The opposite evolution of the effects of exercise on circulating leukocytes between the two groups of subjects could be explained by anemia and / or mild leukopenia observed in the group of anemic athletes. Physical activity alters the immune system in several ways<sup>[28]</sup>. The results of our work confirm this hypothesis. The individual analysis of the types of leukocytes following the specific training program submitted to the participants of this study shows that the amounts of basophils, eosinophils, lymphocytes and monocytes of anemic athletes vary in the opposite direction to those of the group with normal blood counts. Only the neutrophils of the two groups evolve in the same direction by an increase in their blood levels (+ 11.17% and + 1.34% respectively in anemic subjects and those with normal hemoglobin). With the increase in the general concentration of leukocytes, it is logical to observe an increase in neutrophils in athletes following an intense test. Neutrophilia is more or less important depending on the speed of the blood flow. The increase in blood neutrophils is linked to the demargination induced by catecholamines<sup>[29]</sup>. At the start of the experiment, both groups of subjects suffered from mild neutropenia with an amount slightly less than 2000 /  $\mu$ l. Under the effect of the training program, the mean neutrophil value normalized to  $2009.00 \pm 580.80$  /  $\mu$ l. Exercise appears to be more beneficial in healthy subjects compared to subjects with anemia; neutrophils being the first responders to the site of inflammation after infectious contact. By analyzing the basophils of the 2 groups of subjects, a decrease of the order of -35% was observed in the anemic subjects, unlike the subjects with normal blood counts in whom the basophils increased by + 9.99%. Despite the difference in variation, it remained non-significant for each group. A sharp decline in these cells could lead to basophilia, the consequences of which would be a decrease in inflammatory reactions and immediate hypersensitivity in the immune response<sup>[29]</sup>. Lymphocytes and monocytes significantly increased in anemic subjects and decreased in subjects with normal blood counts. In general, the effects of exercise on lymphocytes are strongly experimental protocols<sup>[29]</sup>. Lymphopenia following intensive running parallels the drop in their blood concentration<sup>[30, 31]</sup>. Tomasi *et al.*<sup>[31]</sup> had previously reported that following a 50 km competition for men and 20 km for women cross-country skiing, the percentage of B lymphocytes was significantly increases. As Telgenhoff and Renk had already shown<sup>[32]</sup>, intense training prevents a depression of certain immune parameters in certain athletes (the case of lymphocytes, monocytes and neutrophils in anemic subjects, basophils and eosinophils in athletes. healthy who have rather known an increase) or a decrease (case of basophils and eosinophils in anemic sportswomen, lymphocytes and monocytes in subjects with normal blood counts).

## Conclusions

At the end of this study, we noted that handball players before the training period were divided almost similarly into anemic

athletes and athletes with normal blood counts. The observed changes in WBC count in these 2 groups after 2 weeks of training were dependent on pre-training blood status. These results reinforce the urgency of regular biological monitoring in these athletes, whose economic constraints are very often one of the limiting factors.

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