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Biological Follow-up and Evaluation of the Effects of a Training Program Based on Specific Exercises on the Leucocyte Count of a Group of Cameroonian Handballers

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Abstract: The optimization of the health of sportsmen requires taking into account their hematological parameters in biological follow-up. Unfortunately, this is not the case in most Cameroonian sports structures. This study aimed to evaluate the changes in leukocyte parameters induced by a training program based on specific handball exercises in a group of Cameroonian women's handball. Twenty-nine volunteer female handball players, aged 24.75 ± 1.75 years, with a height of 1.65 ± 0.06 m, a weight of 64.47 ± 6.21 kg and a Body Mass Index (BMI) of 23.62 ± 2.46 kg/m² took part in this study. Their leucocyte formula was determined before and after a two weeks training program, consisting of specific handball exercises. A significant increase in the quantities of lymphocytes (1805.68 ± 503.69/µl vs 1982.21 ± 470.43/µl, $p < 0.05$) was observed. With respective increases of 4.75% and 13.93%, neutrophils (1885.37 ± 554.78/µl vs 1975.10 ± 589.10 ± 84) and basophils (22.00 ± 10.32 /µl vs 25.00 ± 11.78/µl) showed no significant variation. Monocytes and eosinophils also showed no significant decrease at the end of the program, going from 289.07 ± 116.50/µl to 225.92 ± 114.77/µl and from 135.20 ± 82.64 /µl to 99.84 ± 65.98/µl respectively. These results suggest that the exercises to which the handball players were subjected and identical to those carried out during the competitions, led to modifications of the leukocyte count which could have a negative impact on the performance of these handball players in case if their biological follow-up is neglected.

Keywords: Biological Follow-up, Leukocyte Count, Training, Handball

1. Introduction

The immune system protects the body against attacks by infectious agents. It protects in particular against the most classic infections that affect the upper respiratory tract. Regular physical activity is strongly associated with a decrease in the frequency of upper respiratory tract infections [1]. It is not uncommon to find that well-trained athletes have such inconveniences. These phenomena are more troublesome as they oftenly occur during competitions, when the athlete is in his best sport form. However, many studies have shown that after an intense and long effort, the immune system undergoes several modifications which make it less effective against attacks, up to four days after the end of the effort [2].

The effects of physical exercise on the immune system are extremely controversial and diverse. For some, exercise is accompanied by an increase in the number and activity of immune cells [3]. For others, it would have no effects [4]. A third group of scientists claim that physical activity causes a decrease in the immune response [5].

Through these contradictions, one could think that:

1) the measurements taken, only have access to the cells circulating in the blood, which represents less than 10% of the body's reserves;
2) all the cells making up the immune system do not have the same behavior to physical effort and some decreases while others increases;
3) low-intensity physical effort will tend to increase the number and activity of immune cells measured, while intensive training will tend to reduce these parameters.

The sometimes discordant results from one study to another can also be explained by the type of physical activity (intensity and duration), the nature and sensitivity of the test used for the analysis of the immunological parameters.

The intense, repeated and regular physical efforts provided during training and over several years can also lead to metabolic disturbances. High-level sport is marked by the repetition of intense training sessions followed by short recovery periods, which would lead to an increase in energy expenditure. This increase promotes the activation of new metabolic pathways and therefore the hormonal variations of the formed elements of the blood [6].

Physical training and conditioning have been much less studied than acute exercise. Indeed, it is often difficult to get highly trained athletes to stop their daily training program beyond 24 hours, in order to follow-up the future of their hematological parameters (immunological constants on their health chart).

To this end, the available longitudinal studies carried out in these patients are limited by a follow-up period which did not exceed 16 weeks. These studies focused on walking programs for elderly women or women with arthritis and therefore cannot be compared to programs developed for high-level athletes.

High-level sport requires health, clinical, chemical, psychological, technical requirements, etc. Most coaches subject athletes to physical exercises without knowing their health status. The individualized care of an athlete makes it possible to optimize his performance and to detect his health problems early. Biological and hematological follow-up of athletes is rare or even non-existent in Cameroon. However, it is essential for the evaluation of tolerance to training, screening, diagnosis of states of overtraining and doping. In France, the biological follow-up of athletes was instituted in 1999 as part of the policy of protecting the health of athletes and preventing doping.

Several studies have analyzed the responses of hematological parameters to physical exercise.

The results of Ama Moor VJ et al. [7] on the biological follow-up of a group of Cameroonian professional soccer players from the MTN Elite One League, showed significant decreases in hemoglobin concentration and hematocrit ($p<0.05$). They concluded that there was a possible decrease in the fixation of oxygen (O_2) and consequently a decrease in aerobic endurance, one of the consequences of which would be a decrease in the physical performance of the participants in their study. The study of Sylvia AS et al. [8] based on hematological parameter responses and anaerobic capacity through a 12-week training program of Brazilian soccer players reported increases in hemoglobin and hematocrit between the first and the sixth week. These increases could be explained by a reduction in plasma volume resulting from the specificities of predominantly aerobic exercises in football. Michels [9] had reported hematological and manifestations changes following physical exercise in Western athletes. However, in the literature, no similar study has been reported in hematological data in high-level handball training, moreover in the Cameroonian environment, hence this study, whose objective is to analyze the leukocyte count for National Institute and Youth and Sports (NIYS) handball players following a specific training program. For this to be done, the participants underwent blood samples for a Complete Blood Count (CBC) before and after the training program based on specific handball exercises lasting two weeks.

2. Methods

The study is cross-sectional, quasi-experimental. It was carried out at the NIYS in Yaoundé for 2 weeks, with reproductive experiments and in fully controlled experimental environments.

Twenty-nine (29) players from the NIYS handball team took part in this study. The selection of participants was made according to previously defined inclusion and exclusion criteria.

2.1. Inclusion Criteria

To be included in the study, subjects had to:

1) be NIYS students;
2) be aged 27 years maximum;
3) be a handball player for the NIYS team;
4) have regularly participated in the physical and sports training program;
5) have taken part in the two laboratory tests;
6) have responded to the informed consent.

2.2. Exclusion Criteria

Were excluded from our study, handball players who:

1) during the time of the experiment had their period or had become pregnant;
2) had passed the age of 27;
3) did not respond to the informed consent;
4) have been absent from at least one blood sampling session or at least one training program session.

2.3. Equipment for Measuring Anthropometric Parameters

A rigid and removable GM-type measuring rod graduated to the nearest 0.1 cm, from 0 to 250 cm was used to measure standing height. A Yamato brand mechanical scale made in Japan and calibrated to 0.2 kg was used to estimate body weight.

2.3.1. Laboratory Equipment

The Complete Blood Count was done using the following sampling and analysis equipment:

1) cathyyougo brand sterile needles;
2) 5ml glass tubes with an EDTA anticoagulant from Jian Fu Médical, made in Japan in 2015;
3) a blood stirrer;
4) a Sysmex-xs 1000i spectrophotometer from Sysmex Corporation, manufactured in Japan in February 2010.

2.3.2. Measurement Methods and Experimental Protocol

The experimental protocol took place at the NIYS for taking anthropometric parameters on the one hand, and at the Human Biology Laboratory of the Institute for Medical Research and the Study of Medical Plants of the Ministry of Scientific Research and Innovation of Yaounde, for taking hematological parameters on the other hand. The experiment was carried out in 4 steps.

2.3.3. Step 1: Measurement of Anthropometric Parameters

For the measurement of body weight, the player stood on the scale in light clothing, bare feet. The reading of the body mass was done directly on the dial of the scales. For the measurement of the standing height, the subject was barefoot and together, the arms along the body, the gaze horizontal. The experimenter slid the square of the measuring rod which he placed above the subject's head. The reading was done on the amount of the height gauge at the level of the lower base of the square. The Body Mass Index (BMI) was calculated for each participant according to the [10], by dividing the body mass (kg) of each subject by the square of their standing height (m).

2.3.4. Step 2: Blood Collection and Complete Blood Count I

The various hematological parameters of the sportswomen were determined following a CBC, carried out on a multiparametric automaton of the brand HUMACOUNT (WESTBADEN-GERMANY). The samples were taken by a nurse and in the presence of a referring biologist. The athletes were on an empty stomach and the assessment was carried out in the morning, at 8 am. No training had been offered to the players the day before the samples were taken, which were carried out after checking the identity of the subjects. The tubes were labeled at the time of collection. During this phase, the player was seated. The sample was taken following the identification of a vein visible at the elbow crease. A tourniquet was placed upstream on the biceps brachii at the level of the lower 1/3. The sampling area was earlier cleaned using an alcohol swab. The leukocyte count (neutrophils, eosinophils, basophils, lymphocytes, monocytes), the hematocrit level, the hemoglobin level, the Mean Globular Volume (MGV) of the participants were thus measured. For each subject, two samples were taken: the first, the day before the training program. The second collection 24 hours after the program. During this period, no handball player was under contraceptive treatment, nor in menstrual period.

2.3.5. Step 3: Training Program Including Specific Exercises

The specific training program administered to the athletes lasted two (02) weeks. The training sessions were daily and lasted 1 heure 30 minutes.

This program consisted of performing exercises whose intensity and volume were close to those of competitive matches. It consisted of:

1) intermittent runs (30-30 and 15-15), i.e. 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);
2) games with the ball in numerical inferiority;
3) plyometric exercises;
4) multi-hop;
5) counter-attacks (at 2, at 3);
6) duels in attack situation (one against one, three against two, etc.);
7) duels in a defensive situation;
8) specific exercises on goalkeeper parad;
9) relationship games of 2 or 3 in an offensive and defensive situation;
10) games in numerical inferiority on the whole field.

2.3.6. Step 4: Blood Collection and Complete Blood Count II

It made it possible to analyze the various modifications of the parameters of the immune system following the training program administered to the players. The same material and the same procedure as in stage I were used and applied.

Data were presented in tabular form with means ± standard deviation. The analysis was done with graph pad 5.03 software. The even T test was used for the comparison of the beginning and ending means for each parameter. The results of different classes of leukocytes were expressed in microliter and in percentage. The significance level was set at 5% ($P<0.05$). The calculation of the rate of variation of the leukocyte parameters was made according to the following formula: T (%) = Cf-Ci /Ci x100 with T: rate of variation 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.

3. Results

3.1. Anthropometric Characteristics of the Participants

Table 1. *Average values of the anthropometrics parameters of the participants.*

Anthropometrics parameters	**n= 29**
	Mean ± SD
Age (years)	24,7±1,7
Body mass (kg)	64,4±6,2
Height (m)	1,6±0,06
BMI (kg/m²)	23,6±2,4

Age: years; kg: kilogram: kg/m²: kilogram per square meter; n: number of participants; BMI: Body Mass Index; SD: standard deviation.

Table 1 shows that the average age of the twenty-nine (29) female handball players who took part in our study is 24.7 ± 1.7 years, they have an average body mass of 64.4 ± 6.2 kg, a standing height of an average of 1.65 ± 0.06 m and a Body Mass Index (BMI) of 23.6 ± 2.4 kg/m².

3.2. Hematological Parameters Before and After the Training Program White Blood Cells (Leukocytes)

Table 2. *Variation in the level of white blood cells of the players between the start and the end of the training program.*

	NB: usual value between 4-10 x 10^3/µl				
n= 29	Before x 10^3/µl		After x 10^3/µl	T (%)	P-value
	Mean ± SD	4,0±0,9	4,3±0,	6,9%	0,1959 (NS)

n: number of participants; SD: standard deviation; µl: microliter; %: percentage; T: rate of change.

It can be seen from table 2 above that no significant difference in the white blood cell count was observed after the training program, despite a relative increase of 6.9%.

Table 3. *Summary of the variations in quantity (/µl) of the types of leukocytes of handball players.*

	Types of leukocytes	Amount mean (/µl) ± SD	Amount mean (/µl) ± SD	T (%)	P-value
		before	after		
n= 29	Lymphocytes	1805,6±503,6	1982,3±470,4	+9,7	0,0181*
	Monocytes	289,0±116,5	255,9±114,7	-11,7	0,1424
	Neutrophils	1885,3±554,7	1975,1±589,8	+4,7	0,4439
	Eosinophils	135,2±82,6	99,8±65,9	-26,1	0,1043
	Basophils	22,0±10,3	25,0±11,7	+13,6	0,3434

(-) falling; (+) increasing; µl: microliter; %: rate; *: significant difference; P: significance level; T: rate of change; SD: standard deviation; n: number of participants.

Table 3 shows that there is a significant difference in the quantity of lymphocytes (p<0.05). The average is 1805.6 ± 503.6/µl with a percentage of 43.7 ± 7.7% before the training program against 1982.3 ± 470.4/µl with a percentage of 45.4 ± 7.1% after. The usual value is between 20-40%. No statistically significant difference was observed (p>0.05) in the amount of monocytes. The same observation was made for the quantity of eosinophils between the start and the end of the training program (135.2± 82.6/µl vs 99.8±65.9/µl).

Table 4. *Summary of variations in the rate (%) of leukocyte types of handball players.*

	Types of leucocytes	Rate (%) ± SD	Rate (%) ± SD	T (%)	P-value
		Before	After		
n=29	Lymphocytes	43.7 ± 7.7	45.4±7.1	+3.9	0.3119
	Monocytes	7.2 ± 2.1	6.0±2.4	-17.0	0.0251*
	Neutrophils	45,5±8.9	45.5±8.8	+0.06	0.9889
	Eosinophils	3,4±2.4	2,2±1.3	-26.1	0.0390*
	Basophils	0,4±0.2	0.5±0.2	+20.9	0.1823

(-) falling; (+) increasing; µl: microliter; %: rate; *: significant difference; P value: significance level; T: rate of change; SD: standard deviation; n: number of participants.

From Table 4, we observe that there is no significant difference in the rate of lymphocytes between the beginning and the end of the training program (P=0.3119). The level of monocytes of handball players drops significantly between the beginning and the end of the training program (7.2 ± 2.1% vs 6.0 ± 2.4%). No significant difference in neutrophil count from neutrophil count was observed (P=0.9889).

Analysis of Tables 3 and 4 shows that there is no statistically significant difference in the quantity and level of basophils during the experiment despite their increase. Before the training program, the quantity of basophils was 22 ± 10.3/µl and 25 ± 11.3/µl afterwards, for a rate of increase of 20.9%.

4. Discussion

The aim of the study was to analyze the effect of a training program on the leukocyte count of handball players from the NIYS of Yaoundé. We carried out a CBC in these sportswomen before and after the training program composed of specific handball exercises. The CBC made it possible to measure the various hematological parameters.

The characteristics of the various anthropometric parameters of the 29 participants reported an age of 24.7 ± 1.7 years, a standing height of 1.6 ± 0.06 m and a BMI of 23.6 ± 2.4 kg/m². This last parameter, according to [11] is therefore normal because normal subjects have a BMI of between 18.5 and 24.9 kg/m². The study by Ama Moor VJ et al.[7], on the biological follow-up data of a group of Cameroonian footballers from the "MTN elite One league" whose age was 20.7 ± 3.2 years, weight 73.5 ± 5.9 kg and BMI of 23.0±1.3 kg/m² showed significant decreases in hemoglobin concentration and hematocrit ($p<0.05$).

The anthropometric characteristics of the sportsmen in our study cannot be compared to those of the study by Ama Moor VJ et al. [7] because they are subjects of different sexes and practicing a different sporting activity. However, the nature of the physical activity practiced and its intensity influences the blood parameters of athletes differently. Thus, unlike Telgenhoff G and Renk, C [12], who reported that intense training prevents a depression of certain immune parameters in some athletes, our results showed that some of these variables may undergo an increase (case of lymphocytes and basophils) or else a decrease (case of monocytes and eosinophils). Our results corroborate those of Nemet D et al. [13], who also indicated that the absolute number of neutrophils increases during and after acute exercise, since we observed a rate of increase in neutrophils of 4.7%. Despite this increase, no statistically significant difference was observed either in terms of percentage or quantity ($p>0.05$).

The usual value of neutrophils being between 50-80%, the female handball players who participated in our study had a stable neutrophil level below this range, both before (45.5±8.9%) and after (45.5±8.8%) the training program ($p>0.05$). However, the work of Pyne DB et al. [14], reported a decrease in neutrophil oxidative activity after an intense 12-week training program imposed on 12 national-level swimmers.

Walsh et al. [15] showed that prolonged bouts of strenuous exercise cause a temporary drop in white blood cell function and that these changes create a “window” favorable to a drop in protection during which viruses and bacteria can take hold, thus increasing the risk of developing an infection. During the training program submitted to the handball players, none of them fell ill despite the decrease in certain types of leukocytes such as monocytes and eosinophils. The decline in immune defenses remains temporary and lasts a maximum of 3 to 72 hours, following the end of the exercise period [16]. The decrease in the functions of T lymphocytes, following an intensive race, is parallel to the drop in their concentration in the blood [17].

The indices of immune function in athletes in a state of complete rest (24 hours after the last session) are not different from those of sedentary ones, except if the athletes are engaged in periods of intensive training. In this case, immune function may not be completely influenced by successive exercise sessions and some of its elements may chronically decrease [18]. Our subjects underwent the second CBC 24 hours after the training program and had sufficient recovery periods to avoid overtraining. Contrary to the decline in neutrophils whose usual values are below normal, eosinophil levels remain within the normal range (1-4 x $10^3/\mu l$). Before the training program, this rate was 3.4 ± 2.4% against 2.2 ± 1.3% after the training program.

Lewicki et al. [5] also reported a decrease in the immune response during physical activity. The work of Pedersen BK and Bruunsgaard H [3] showed that physical exercise is accompanied by an increase in the number and activity of immune cells. The analysis of our data showed increases in the quantities of lymphocytes, neutrophils, basophils with rates of increase of 9.78%, 4.75% and 13.63% respectively. All the elements of the leukocyte formula of our subjects underwent variations, either by increase or by decrease. The immune vulnerability induced by the succession of intense physical exercises requires vigilance from the coach or physical trainer in supporting athletes [19].

Physical activity and sport are at the origin of a disturbance of homeostasis with a systemic response of the inflammatory type which, depends on parameters such as age, sex, level of training, intensity., duration of exercise, nutritional status, lifestyle or even genetic factors [9]. The training program submitted to the handball players who constituted our study population effectively led to a hematological disturbance as evidenced by the different variations of the leukocyte count.

5. Conclusion

The objective of this study was to analyze the effects of a training program based on specific exercises on the leukocyte formula of handball women from the National Institute of Youth and Sports of Yaoundé. At the end of this study, we noted that the training program submitted to our subjects led to various immune disturbances. Some leukocyte parameters underwent increases (lymphocytes and neutrophils) and others, on the other hand, experienced decreases (monocytes and eosinophils). These recorded leukocyte disturbances could have a negative impact on the performance of these handball players in the event of negligence in their biological follow-up.

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