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Evaluation of Nutritional, Hematological and Pathological Profiles of Congolese Endurance Athletes during Competition

Eddie Janvier Bouhika[1,2,3,*], Paul Roger Mabounda Kounga[2,4], Florent Nsompi[2,5], Simplice Innocent Moussouami[2,5], Alain Marc Boussana[1,2], Jean Martin Moussoki[2,6], Denis Mbaïnaïssem[3], Michel Elenga[3], Vital Mananga[3], Yvon Simplice Itoua Okouango[3], Etienne Nguimbi[6], François Mbemba[1,3]

[1]Nutrition, Health and Human Motricity Research Unit, Higher Institute of Physical Education and Sports, Marien NGOUABI University, Brazzaville, BP: 69, Republic of Congo
[2]Education, Health, Expertise and Optimization of Motor Performance Research Unit, Marien Ngouabi University, Brazzaville, BP: 69, Republic of Congo
[3]Nutrition and Human Food Laboratory (LaNAH), Faculty of Science and Technology (FST), UMNG, BP: 69, Brazzaville, Congo
[4]Molecular Biology Laboratory, Shanghai Sports University (China)
[5]Sport, Health and Evaluation Research Unit (UR/SSE). National Institute of Youth, Physical Education and Sport (INJEPS), University of Abomey-Calavi (UAC). 01 BP: 169. Porto-Novo, Benin
[6]Cellular and Molecular Biology Laboratory of the Faculty of Science and Technology, Marien NGOUABI University, BP: 69, Brazzaville, Republic of Congo
*Corresponding author: eddie.bouhika@umng.cg

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Abstract **Context:** The monitoring of eating habits through the biological data of athletes contributes to the preservation of the athlete's health. **The objective** of this study was to evaluate the nutritional state, the hematological profile and the pathological state of 64 Congolese endurance athletes (32 athletes or experimental group and 32 walkers or control group) respectively aged 26.16 ± 2.79 years and 27.44 ± 3.44 years. Data were compared using chi^2 and student’s “t” test for independent-samples. **Method:** the body mass index and the hematological examination made it possible to evaluate the nutritional state, the blood parameters and the pathological state of the respondents. **Results:** the respondents were of normal weight, but cases of light and moderate thinness were observed in the athletes (18.75% and 6.25%). Hematological examination of the subjects showed that levels of leukocytes (4.64±0.93x10^3/mm^3 vs 7.12±3.07 x10^3/mm^3), hemoglobin (12.42±1.80g/dl vs 11.29±1.96 g/dl) and thrombocytes (202.25 ±51.89 x 10^3/mm^3 vs 307.47±28.64 x 10^3/mm^3) was normal in both groups but a little high in walkers. The pathologies found in the two groups were anemia (46.88% vs 71.88%), leucopenia (12.5% vs 0%) and bicytopenia (9.38% vs 9.37%). However, anemia was more marked in walkers. Leukopenia was observed only in athletes, and bicytopenia was observed in both groups. **Conclusion:** the nutritional data were mostly normal but the hematological data were abnormal with the presence of pathologies which are often associated with poor performance and which deserve to be corrected by a balanced and controlled diet.

Keywords: *eating habits, nutritional status, Hematological examination, nutritional pathologies, endurance athletes, competition*

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1. Introduction

In the past, an athlete's sporting success was determined by the harmonious relationship between him and his coach, despite the latter's knowledge. Subsequently, many studies have highlighted the determining role of food for physical performance [1]. These foods provide nutrients that are primary fuels during intense physical exercise [2].

In addition, to improve performance also requires a balanced diet since the determining role of carbohydrate diet on muscle glycogen content, and therefore on maximal aerobic endurance has been demonstrated [3,4].

In addition, protein food intake is encouraged especially in athletics events in order to gain muscle mass [5].

As a result, physical activity requires an appropriate diet. Athletes must benefit from a quantity of food to meet the needs for energy, quality, macronutrients (carbohydrates, lipids, proteins), micronutrients (vitamins and mineral salts) and water [6].

A good food choice offers many benefits to all athletes, regardless of event, age, or level of competition [7]. These benefits include optimal and normal nutritional status, reduced risk of injury and disease. Despite these benefits, many athletes do not meet their dietary needs. It is recognized that athletes who train hard or travel and compete regularly may be more frequently exposed to minor illnesses and infections [8].

The biological assessment as a tool to objectify the feelings of athletes, prevent deficiencies and excesses, help to personalize the level of training. It is also used to detect abnormalities in the lipid balance and can allow athletes to adapt their diet [9].

For example, the "blood sugar level" is fundamental for the athlete. Its stability is essential at all stages of training or competition. Indeed, hypoglycaemia severely limits an athlete's abilities. Similarly, the Complete Blood Count is used as a general screening test to look for disorders such as hemolytic, deficiency, inflammatory abnormalities, infections etc… [10].

Most of us who practice sports, we stray from healthy sports quite often. This is why it is so important to have a medical follow-up. Thus, the blood test will allow us, through the doctor's interpretation, to diagnose a possible pathology or to suspect nutritional deficiencies [11].

In Congo Brazzaville, the athletes participating in the 11th African Games, organized in Brazzaville from September 4 to 19, 2015, were characterized by poor performance and even dropouts in endurance races [12]. This context leads us to verify the biological and hematological profile of middle distance and long distance runners in preparation for the African Games. In other words, it is a question of unsealing the lipid profile of Congolese middle-distance and long-distance runners during competition.

Many studies have linked the nutritional habits and performance of endurance athletes [13], the training conditions and dietary situation of Congolese athletes , the prevalence of carbohydrates in endurance runners [14]. However, few studies address the dietary profile in relation to the hematological data of Congolese athletes [15].

The Republic of Congo is distinguished by a constant deterioration of living conditions, resulting in the undernourishment of the general population. [16]. This could deteriorate the hematological and biochemical data of individuals who practice sports [17].

It is in this way that several works have examined the impact of a healthy and balanced diet on the performance achieved during a regular practice of physical and sporting activity, both from a quantitative and qualitative point of view [18,19].

In Congo-Brazzaville, concerning the diet of athletes, few studies have been carried out [20]. Thus, to our knowledge, no study has been interested in the evaluation of the hematological, biochemical profile and in order to list the nutritional pathologies of Congolese athletes in pre-competition and competition periods, in athletes practicing endurance events, in comparison with practicing subjects dedicated in sport walking.

Knowing that, a varied and balanced diet is necessary to meet the energy needs of the athlete, it must then be based largely on the choice of balanced foods [21]. This diversified and regular diet is sought by anyone wishing to maintain balance, physical fitness, a good nutritional and hematological state that does not include pathologies or nutritional anomalies.

However, in Congo, the biological monitoring of athletes is almost rare, even non-existent. It is realized in need to get the license established without a proper medical examination. It is therefore difficult to detect some pathologies in athletes from the start of their career or at the start of each sports season. However, simple clinical visits are considered an important act to obtain a sports license without prior biochemical and hematological examination.

To this end, the question that arises is whether the hematological profile of middle-distance and long-distance athletes during the competitive period is likely to explain the poor performance observed since the eleventh African Games?

This work aims to assess the biological data of athletes during the competition period, to be compared with the data of athletes who practice sport walking.

2. Methodology

2.1. Participants

We conducted the study among the male and female sports population of Brazzaville practicing endurance races in preparation for national and international middle-distance (MD) and long-distance (LD) competitions of the 2012-2016 Olympiad. And the one in progress, and all the sport walkers of the city of Brazzaville. The non-random method and the reasoned-choice technique allowed us to retain a sample of 64 subjects including 32 long-distance (LD) and middle-distance (MD) athletes and 32 walkers practicing sport walking. The subjects were divided into two groups: an experimental group (32 trained athletes, i.e. 22 boys and 10 girls) practicing endurance races (MD and LD) whose average age is 26.16 ± 2.79 years, an average height of 1.69±0.07m, an average weight of 57.28±4.49kg and a BMI of 19.73±1.49kg/m^2. A control group made up (32 Sport walkers, i.e. 22 boys and 10 girls) whose average age is 27.44 ± 3.34 years, an average height of 1.66 ± 0.04 m, an average weight of 63.13 ± 4.03 kg and a BMI of 22.49±1.50 kg/ m^2.

2.2. Experimental Protocol

The investigation took place in three phases. First of all, the first two days were devoted to measuring the height, weight and collecting information related to the social status of the respondents. Then we submitted a questionnaire to the 32 athletes and 32 Sport walkers relating to the food survey. Data on the various aspects related to food behavior were collected using food survey forms [22] which were filled out on site and collected by the interviewer. In the end, the trained athletes and the sport walkers were received at the national laboratory for the blood sample which had been taken in 4 days. Blood samples for the two groups (elite athletes *vs* sport walker club) were taken by qualified personnel from the National Public Health Laboratory. All these analyzes were carried out via an automatic process of the biochemistry laboratory at the National Public Health Laboratory of Brazzaville.

2.3. Determination of Hematological Parameters

Biomarker examinations such as Complete Blood Count (CBC) were carried out in the subjects of the experimental group (EG) and the control group (CG) at the National Public Health Laboratory of the Republic of Congo, precisely at the Direction of Medical Biology in Brazzaville.

These samples were taken in the morning on an empty stomach. Blood was collected in dry sterile vacuum tubes bearing the subject's name or number. The blood samples were centrifuged (3500 rpm, 15 minutes) and the collected sera were stored at -20°C. [23]. These Hematological examinations should make it possible to diagnose some possible pathologies and to suspect nutritional deficiencies [24].

2.4. Equipment Used

Biological material: human blood

Laboratory equipment: sterile single-use needles (21G) for blood sampling; micro infusion sets for blood sampling, tourniquets, 90° alcohol, sterile gloves (single use); absorbent cotton; hemolysis tubes; sticking plaster; indelible ink markers; a red blood cell counter (PENTRA ES 60), racks for transporting the tubes, adjustable micropipettes from 10 to 100 µl and from 500 to 1000100 µl, tips for the serum and for the reagents, a centrifuge, a refrigerator; a freezer ; an HP computer; an HP printer; a spectrophotometer for the analysis of biochemical data (KENZA MAX BioChemisTry); and BIOCHEMISTRY ABALYSTER RX and racks for tube transport)

2.5. Statistical Analysis of Data

For data processing, we used the Statistical Package of Social Science for Windows (SPSS) version 22 software. The frequency, ratio and cross-referencing between quantitative and nominal variables were made from descriptive statistics. To do this, the comparison of the means between two groups was done using chi^2 tests and Student's "t" test for independent samples, and the comparison of the means between two or more groups was done using the analysis of variance test (ANOVA). Quantitative variables are expressed as the mean ± standard deviation (± σ), and qualitative variables as a percentage. The significance threshold was set at $p < 0.05$.

3. Results

The results concerning the anthropometric data (Table 1) indicated that no significant difference was found in age between the two groups. However, we noted significant differences in weight (57.28 ± 4.49 kg vs 63.13 ± 4.03 kg, p<0.000). Height also differed between the two groups (1.69 ± 0.07m vs 1.66 ± 0.04m, p <0.05). Similarly, BMI had a significant difference between the two groups (19.73 ± 1.49 Kg/m² vs 22.49 ± 1.50 Kg/m², p<0.000).

Regarding the classification of the nutritional status (Table 2), the results showed that the nutritional status was normal respectively (75% vs 71.88%) for the experimental group and the control group. However, we found subjects in a state of moderate thinness respectively in 6.25% and 3.12% of the athletes and Sport walkers. A percentage of slight thinness was also found in 18.75% and 3.12% of athletes and walkers, respectively.

Concerning the existence of biological examinations (Table 3), the results showed that 100% of female and male athletes did not benefit from biological examinations at the beginning or in the middle of the sports season. However, biological examinations have been proven beneficial for athletes. What must be required in order to diagnose deficits and some nutritional deficiencies.

With regard to the hematological examinations carried out in the laboratory (Table 5 and 5-1), the results according to the groups (Table 4) and according to the sexes (Tables 5-1 and 5-2) showed that the levels of leukocytes, erythrocytes , hemoglobin and thrombocytes were within the range of norms for both groups. However, we found significant differences in leukocytes (4.64± 0.93 x 10^3 /mm^3 vs 7.12±3.07 x 10^3 /mm^3 respectively in runners and walkers, P < 0.000). For hemoglobin and thrombocytes, the levels were respectively 12.42± 1.80 g/dl vs 11.29±1.96 g/dl and 202.25±51.89 x 10^3 /mm^3 vs 307, 47±28.64 x 10^3 /mm^3 in athletes runners and athletes walkers with a significant difference of P < 0.02 and P < 0.000.

With regard to the pathologies found, the results showed (tables 6-1 and 6-2) the presence of anemia in the two groups (46.88% vs 71.88%), leukopenia in the subjects of the experimental group (12.5%), anemia + leucopenia within the experimental group (3.12%), anemia + bicytopenia at the experimental group (9.37%) and the control group (9.37%). Similarly, according to gender, anemia was marked in 36.4% vs 86.4% respectively in boys in the experimental group and boys in the control group and in 70.0% vs 40.0% respectively in girls from the experimental group and girls from the control group.

Table 1. Anthropometric data of the subjects

Variables	Experimental Group (G=10; B=22)	Control Group (G=10; B=22)	F	signification
	$\bar{x} \pm \sigma$	$\bar{x} \pm \sigma$		
Age (years)	26.16 ± 2.79	27.44 ± 3.34	-1.66	NS
Weight(Kg)	57.28 ±4.49	63.13 ±4.03***	-5.47	<0.000
Size (m)	1.69 ±0.07*	1.66 ± 0.04	2.05	<0.05
BMI (Kg/m²)	19.73 ± 1.49	22.49 ± 1.50***	-7.37	<0.000

Legend : NS = not significant; * : significant ; **: very significant; ***: highly significant, G=Girls, B= Boys.

Table 2. Subject characteristics

variables	Classification of BMI by WHO	Experimental Group (Girls = 10; Boys =22)%	Control Group (Girls= 10; Boys =22)%
Age (years)		26.16 ± 2.79	27.44 ± 3.34
Weight (Kg)		57.28 ±4.49	63.13 ±4.03***
Size (m)		1.69 ±0.07*	1.66 ± 0.04
BMI (Kg/m²)		19.73 ± 1.49	22.49 ± 1.50***
Nutritional state			
Slimming / Severe thinness	< 16.00		
Moderate thinness	16.0-16.99	2(6.25)	1(3.12)
slight thinness	17.0-18.49	6(18.75)	1(3.12)
Normal	18.50-24.99	24(75)	23(71.88)
Overweight	25.50-29.99	7(21.88)	-
Moderate obesity	30.0-34.99		
Severe obesity	35.3-39.99		
Very severe obesity	> 40		

Table 2 shows that the nutritional status was normal (75% vs 71.88%) for EG and CG respectively. However, the two groups did not differ in age. But, the control group differed significantly from the experimental group in weight and BMI, respectively (63.13 ± 4.03 vs 57.28 ± 4.49 kg and 22.49 ± 1.50 vs 19.73 ± 1.49 kg/m²). However, there is a significant height difference in favor of the experimental group (1.69 ± 0.07 vs 1.66 ± 0.04 m).

Table 3. Existence of Biological examinations in EG athletes

Answer	Girls and Boys (N=32)	%
Yes	0	0.00
No	32	100

Legend: EG=Experimental Group.

Table 4. Hematological examinations (Blood count) of the subjects according to the groups

Variables	Experimental Group (G=10; B=22)	Control group (G=10; B=22)	F	Signification
	$\bar{x} \pm \sigma$	$\bar{x} \pm \sigma$		
Leukocytes (x 10^3/mm^3)	4.64±0.93	7.12±3.07***	-4.37	< 0.000
Erythrocyte (x 10^6/mm^3)	4.71±0.62	4.51±0.99	0.98	NS
Hemoglobin (g/dl)	12.42±1.80*	11.29±1.96	2.39	< 0.02
Thrombocytes (x 10^3/mm^3)	202.25±51.89	307.47±28.64***	-10.04	< 0.000

Standard values for girls and boys: Leukocytes (x 10^3/mm^3): Girls and Boys= 4.0 to 10.0; Erythrocytes (x 10^6/mm^3): Girls = 3.80 to 5.80 and Boys = 4.50 to 6.50; Hemoglobin (g/dl): Girls = 11.5 to 16 and Boys = 13 to 17.7; Thrombocytes (x 10^3/mm^3): Girls and Boys = 150 to 450.

Table 5. Comparison of hematological parameters according to sex

Table 5.1. ANOVA

variables		sums of squares	dof	medium square	F	Signification
Leukocytes	Inter group	110.23	3	36.74	7.20	<0.000
	Intra group	306.24	60	5.10		
Erythrocytes	Inter group	4.89	3	1.63	2.55	NS
	Intra group	38.36	60	0.64		
Hemoglobin	Inter group	58.33	3	19.44	6.44	<0.001
	Intra group	181.19	60	3.02		
Thrombocytes	Inter group	265455.71	3	88485.71	257.75	<0.000
	Intra group	20598.03	60	20598.03		

Table 5.2. Multiple comparison of hematology data

Variables	BEG n=22	BCG n=22	GEG n=10	GCG n=10	Signication
Leukocytes (x10^3/mm^3)	4.54 ±0.91	7.52 ±3.32***	/////	/////	<0.000
	/////	7.52 ±3.32**	4.87 ±0.99	/////	<0.003
	/////	/////	4.87 ±0.99	6.23 ±2.32	0.18
Erythrocytes (x10^6/mm^3)	4.95±0.47*	4.46±1.05	/////	/////	<0.05
	4.95±0.47*	/////	4.18±0.58	/////	<0.01
	/////	/////	4.18±0.58	4.62±0.91	0.23
Hgb (g/dl)	13.04±1.58***	10.90±1.80	/////	/////	<0.000
	13.04±1.58**	/////	11.04±1.51	/////	<0.004
	/////	/////	11.04±1.51	12.15±2.11	0.16
Thrombocytes (x 10^3/mm^3)	169.68±16.36	293.55±19.87***	/////	/////	<0.000
	169.68±16.36	/////	273.90±18.83***	/////	<0.000
	169.68±16.36	/////	/////	338.10±19.69***	<0.000
	/////	293.55±19.87**	273.90±18.83	/////	<0.007
	/////	/////	273.90±18.83	338.10±19.69***	<0.000

Legend: BEG = boys in the experimental group; BCG = boys in the control group; GEG = Girls in the experimental group; GCG= Girls in the control group., Hb=Hemoglobin, G=Girl; B=Boys.
Standard values for girls and boys: Leukocytes (x 10^3/mm^3): G and B= 4.0 to 10.0; Erythrocytes (x 10^6/mm^3): G = 3.80 to 5.80 and B = 4.50 to 6.50; Hemoglobin (g/dl): G = 11.5 to 16 and B = 13 to 17.7; Thrombocytes (x 10^3/mm^3): G and B = 150 to 450.

Table 6. Pathologies found

Table 6.1. Pathologies found in the subjects according to the groups

Status	Experimental group n(%)	Control group n(%)	Total N (%)
No anemia (normal)	17 (53.12)	9 (28.12)	26 (40.62)
Anemia	15 (46.88)	23 (71.88)	38 (59.38)
Neither Leuco/ nor bicyto	25 (78.12)	29 (90.63)	54 (84.37)
Leukopenia	4 (12.5)	-	4 (6.25)
Bicytopenia	3 (9.38)	3 (9.37)	6 (9.37)
No anemia-nor leuco/nor bicyto	14 (43.75)	9 (28.12)	23 (35.94)
Anemia - neither leuco/ nor bicyto	11 (34.38)	20 (62.5)	31(48.44)
No anemia + Leukopenia	3 (9.37)	-	3 (4.69)
Anemia + leukopenia	1 (3.12)	-	1 (1.56)
Anemia + Bicytopenia	3 (9.37)	3 (9.37)	6 (9.37)

NB: Neither leuco/nor bicyto: normal subject not showing leukopenia and bicytopenia.

Tables 6.2 Prevalence of pathologies in subjects according to gender

Sex	Normal n (%)	Anemia n (%)	Total N (%)
BEG	14 (63.6)	8 (36.4)	22 (100)
BCG	3 (13.6)	19 (86.4)	22 (100)
GEG	3 (30.0)	7 (70.0)	10 (100)
GCG	6 (60.0)	4 (40.0)	10 (100)

Legend: BEG: Boy of Experimental Group, BCG: Boy of Control Group, GEG: Girl of Experimental Group, GCG: Girl of Control Group.

4. Discussion

The objective of this study was to analyze the nutritional status, hematological parameters and the prevalence of pathologies in Congolese athletes and walkers. However, making choice about what to eat and how much to eat requires knowledge of nutrition [25].

The main results on the nutritional status, the biological data and the pathologies of the subjects were as follows:

Nutritional status of subjects

The results of this study show that the anthropometric data of the subjects surveyed (athletes and walkers) were not significant concerning the age. However, we noted a significant difference in weight ($p<0.000$), height ($p<0.05$), and BMI ($p<0.000$). The nutritional status of the subjects surveyed was mostly normal (ie 75% in EG of athletes and 71.88% in CG of sport walkers) (Table 1 & Table 2). Similar work was conducted by Baranauskas et *al*., [21], with Lithuanian endurance athletes. The results of this study showed that the majority of respondents were within the normal body weight range (with a BMI that was between 19 and 24 kg/m^2). Our results also agree with those found by Bouhika et *al* ., [26] on the energy intake of Congolese middle-distance athletes during competition. The results of this study had shown that 73.33% of male athletes and 100% of female athletes had a normal nutritional status.

The biological data and the pathologies of the subjects

The determination of the carbohydrate, lipid and protein balance was made by means of a blood sample.

The results of this study indicates that the athletes did not have the culture to do the biological examinations (biochemical and hematological). Because, 100% of the subjects of the EG had answered "no" to the existence of Biological examinations. However, these examinations are very important to know the state of health of an athlete during the sports season. Biological examinations play a very important role in diagnosing a possible pathology in the athlete, analyzing the physio-pathological impact of intense sports practice on the individual, verifying the relevance of training, preventing the deleterious effects of poor nutrition adapted, including eating disorders (ED), which can contribute to the appearance of a state of overtraining or dys-training [27]. The results of these examinations can make it possible to offer nutritional advice adapted to the needs of the sports specialty. The examinations can be personalized to respect the specificities of each athlete and can help partially detect illicit practices during the preparation of athletes [28]. The results of this study showed that the athletes of the experimental group were never subjected to biological examinations (biochemical and hematological) (n=32 or 100%) (Table 3). However, according to Rieu [29], medical examinations are a very important act for athletes in order to know not only the state of health of the practitioner but also to protect him from some dietary needs, pathologies caused by certain forms of sports practice, the physiological changes linked to various types of training, the body's adaptation to effort and the effects of sport on growth, aging, fertility, the heart, blood vessels and joints.

These examinations are important in sports medicine care. In France, for example, biological monitoring of athletes was instituted in 1999 as part of the policy to protect the health of athletes and prevent doping [30] . Indeed, these examinations, which are very useful for detecting dietary errors in training and competition, are neglected by many club managers, who are unaware of the importance of an annual basic dietary assessment to control biochemical parameters (glycaemia, serum creatinine, triglycerides, cholesterol like total LDL and HDL) and hematological parameters (leukocytes, erythrocytes, hemoglobin and thrombocytes) [31].

The hematological results showed that the level of erythrocytes was slightly elevated while that of hemoglobin was higher in the subjects of the experimental group. In addition, leukocyte and thrombocyte levels were significantly elevated. Taking gender into account, on the one hand, the erythrocyte and hemoglobin levels of the boys in the experimental group were very high, while those of the girls in the experimental group were low. On the other hand, leukocyte and thrombocyte levels were significantly elevated in boys and girls in the control group. These results also showed that the athletes suffered from pathologies such as anemia, leukopenia and Bicytopenia.

With regard to hematological parameters, it should be noted that physical exercise today plays a very important role in the prevention and resistance of the body against some microbes. Regular moderate-intensity training has beneficial effects on the immune system and excessive exercise can have negative consequences [32]. However, if physical activity favorably influences the immune system, it should be noted that the effects of chronic physical training on these cells are not well known. According to the study by Horn et *al*. [33] conducted on a large sample of more than 1000 elite male athletes, over a 10-year period in 14 different sports disciplines, athletes who practiced individual aerobic sports such as cycling and triathlon had lower leukocyte levels lower.

Similarly, according to data from longitudinal and cross-sectional studies, endurance-type athletes seem to have lower total leukocyte levels and that aerobic training can decrease leukocyte counts [34]. These results are in line with ours. Compared to subjects in the control group, subjects in the experimental group showed significantly low leukocyte levels (7.12 ± 3.07x10^3/mm^3 *vs.* 4.64 ± 0.93 x 10^3/mm^3; t=-4.37; p<0.000) (Table 4). Theses results are in agreement with the work of Saygin et *al*. [35] who, after comparing leukocyte values in sedentary individuals, volleyball players, and running athletes, reported higher total leukocyte counts in volleyball players, followed by sedentary individuals. However, in endurance athletes, these same authors obtained a lower number of leukocytes. Several studies have reported that long-term endurance exercise has a negative impact on immune function [36].

This study showed that compared to subjects in the control group, boys in the experimental group had low leukocyte counts (4.54 ± 0.91 x10^3/mm^3 *vs* 7.52 ± 3.32 x10^3/mm^3; p < 0.000). This drop in leukocytes can be explained by the fact that prolonged exercise increases circulating stress hormones (adrenaline, cortisol, etc.) which act on all leukocytes. Similarly, prolonged exercise deteriorates the balance between pro- and anti-inflammatory cytokines [37]. Furthermore, Gleeson et *al* . [38] reported that inadequate or inappropriate nutritional intake was at the root of the deterioration of immune function in endurance athletes.

Concerning erythrocytes, note that they are mainly known for their role in the transport of respiratory gases (O^2 and CO^2). Besides that, they perform a variety of other functions, which can also improve physical performance. Erythrocytes, for example, absorb the lactate released by muscle cells during high-intensity exercise and decrease the plasma concentration of metabolites. Our results show that, compared to the control group, the experimental group showed slightly elevated erythrocyte (ER) levels but did not show a significant difference (4.51 ± 0.99 x10^6/mm^3 vs 4.71 ± 0.62 x10^6/mm^3; t = -0.98; p > 0.05) (Table 4).

The results of this study are in agreement with those found by Mairbäurl [37] who reported an increase in blood volume, blood plasma and RBC level in middle distance and long distance athletes during intense exercise. However, in female (middle-distance) athletes, blood volume was still elevated while blood plasma and RBC levels were lower compared to male long-distance athletes. This drop in RBCs in cross-country skiers is similar to that observed in our daughter athletes in the experimental group. Compared to the boys in the experimental group (BEG), the girls in the experimental group (GEG) showed significantly low RB levels (4.95 ± 0.47 x10^6/mm^3 *vs.* 4.18 ± 0.58 x10^6/ mm^3 ; p<0.05) (Table 4). The drop of RBC levels in girls of the experimental group can be explained by a diet low in iron, haemolysis, menstruation, mechanical constraints, type of activity, contact of the foot on the ground, etc. [39]. This decrease of erythrocytes in the girls of the experimental group is explained by sports anemia. The results show that 7 out of 10 girls, or 70%, had anemia, while 8 out of 22, or 36.4%, of the boys in the experimental group also had it. This anemic condition was also present in 86.4% of boys in the control group (Table 6-2). This is probably due to insufficient protein intake and a diet low in iron. These results support studies by Yoshimura et *al*. [40] and Hunding et *al*. [41] who had observed an alteration of erythrocytes after insufficient protein and iron intake.

With regard to hemoglobin, note that the impact of exercise alone on its increase has not yet been precisely identified. It is generally accepted that regular endurance training regimens cause cardiac and hematological adaptations. Many work has indicated that total hemoglobin varies in different elite endurance athletes. This is no doubt due to a number of factors, in particular the specificity of the sport, the level of physical preparation of the athletes, the age of the subjects, the training load and genetic factors [42]. In endurance athletes, hemoglobin is considerably elevated compared to untrained subjects and those with relatively low physical preparation. This statement above is in agreement with the results of our work. Compared to subjects in the control group, subjects of the experimental group showed significantly elevated hemoglobin levels (11.29 ± 1.96 g/dl *vs* 12.42 ± 1.80 g/dl; t = 2 .39; p < 0.05) (Table 4). These results are in agreement with the work of Kratz et *al*. [43] who had reported an increase in hemoglobin in marathon runners. According to Mairbäurl [37], this increase of hemoglobin levels in endurance athletes is explained by an increase of the quantity of oxygen delivered to the tissues. However, the study by AmaMoor et *al*. [30] had shown, during biological monitoring, that football players in Cameroon had low hemoglobin levels. This protein is involved in the transport of oxygen and is very important because higher is the value, better is the transport. But, during training and competition this value can decrease and return to normal.

Hemoglobin is one of the most studied physiological variables related to endurance-induced adaptations. In this study, no significant difference was observed in the girls of the control group compared to the data of the girls of the experimental group. This study corroborates the study by Damien et *al*. [44] and that of Umarani and Shelvam [45] who reported a non-significant difference of hemoglobin in female athletes and non-athletes. This slight drop of hemoglobin compared to normal is due to sports anemia. According to Ottomano and Franchini [46] and Damien et *al.* [44], this anemia can be explained by an iron deficiency caused by an increased nutritional need or loss of iron, by intravascular hemolysis, by an expansion of the blood volume and by a poor nutritional intake. However, our results show a significant difference between boys of the control group and those in the experimental group (13.04 ± 1.58 g/dl *vs* 10.90 ± 1.80 g/dl; $p < 0.000$) (Table 5 - 2). This study is in agreement with the work of Defferrard and Lepori [47] who observed a 6% increase in hemoglobin during training for 9 months. According to these authors, although training for 9 months increased Hb levels, many years of training may be required for a significant increase in Hb levels.

In some athletes, a temporary drop of hemoglobin can be observed at the very beginning of training or a drop of erythrocytes starting from the preparation period until the competition period called hemodilution or sports anemia [37]. The results of this study showed that 46.88% of subjects in the experimental group and 71.88% of subjects in the control group suffered from anemia (Table 6-2). For the athletes of the experimental group, the occurrence of anemia can probably be justified by an adaptation to the effort. In agreement with our results, Larson [48] reported that this sports anemia appeared to be a beneficial adaptation for aerobic training and in no way compromised performance. In the athletes of the control group who were subjected to a low intensity, the presence of anemia can probably be justified by a lack of iron. According to the results of many studies, the anemia often encountered in athletes is partly linked to a lack of iron. However, this lack of iron causes an alteration of muscle function and limits work capacity, which can subsequently compromise training and athletic performance [49].

Regarding thrombocytes, it should be noted that they are blood cells that play an important role in the formation of blood clots. Platelet hyper-activation is implicated in the onset of atherosclerosis, CVD, myocardial infarction and stroke [50]. The increase of thrombocytes could be attributed to contraction of the spleen regulated by a hormone called adrenaline during exercise, which causes the release of thrombocytes into the bloodstream. According to Lippi et *al*. [51] and Alis et *al*. [52] after physical exercise, whether in sedentary subjects or in trained subjects, the levels of thrombocytes decrease 30 minutes after exercise. This decrease can also be observed 3 hours and 20 hours after exercise.

According to the results of our study, the reduction of the level of thrombocytes was very significant in the subjects of the experimental group compared to those of the control group (202.25 ± 51.89 $x10^3$ / mm^3 *vs.* 307.47 ± 28.64 $x10^3/mm^3$; t=-10.04; $p<0.000$) (Table 5). The thrombocyte levels of the subjects were lower (202.25 ± 51.89 x $10^3/mm^3$) than those of the marathon runners reported in the study by Lippi et *al.* [51].

These authors reported that the rates of marathon runners were on average 263 x $10^3/mm^3$ three (03) hours after physical activity and 255 x 10^3 /mm^3 twenty (20) hours after physical activity. This difference in platelet counts is probably due to genetic and racial influence [53]. Looking at the results of thrombocytes in relation to gender, our results show that boys of the experimental group had low levels compared to boys of the control group and girls in both groups respectively. It should be noted that the levels of thrombocytes between the girls in the experimental group and the girls of the control group presented a considerably large difference compared to that which exists between the girls of the experimental group and the boys of the control group. This progressively slow fall of the level of thrombocytes in girls and boys of the control group can no doubt be explained by the practice of irregular and non-intense physical activity. Alis et *al*. [52], for example working on a group of active subjects (12 hours of endurance per week) and another group of sedentary subjects (less than one hour of physical activity per week), had noticed a progressively slow return of the rate thrombocytes in sedentary subjects.

The pathologies of the subjects

In general, regular moderate-intensity training is known to have beneficial effects on immune function, while elite training has detrimental effects on the athlete's health, leading to frequent infectious episodes, including respiratory tract [54]. While white blood cell counts in resting athletes are generally similar to those in the general population, low lymphocyte, neutrophil, and monocyte counts may result from intensive aerobic training [54]. This drop of leukocytes or leukopenia is often encountered in marathon athletes and is therefore encountered in a few athletes in our study. Indeed, our results show that in the experimental group, 12.5% of the subjects suffered from leukopenia; 9.38% of subjects suffered from bicytopenia; 3.12% of subjects suffered from anemia and leucopenia; 9.37% of subjects suffered from anemia and bicytopenia. However, in the control group, no subject showed signs of leukopenia while 9.37% of subjects suffered from leukopenia and 9.37% of subjects suffered from anemia and bicytopenia. This observation of the decrease in white blood cells or leucopenia on the one hand and the decrease in red blood cells on the other hand, is in agreement with other studies carried out in endurance elites, cyclists, swimmers, etc. [55]. These two pathologies can be explained by the practice of intense and specific exercises carried out during training or competition (Table 6).

Study limitations

The sample of our study cannot be considered as representative of the Congolese population. However, it reflects a great diversity of situations and gives a fairly clear idea of the economic and social reality of Congolese athletes.

Random errors are limited by the small size of the sample, but the unique realization of the questionnaire does not escape the variability of the parameters studied.

The non-sampling of athletes' blood after the African Games competition period for the purpose of comparing biochemical data between the two periods was a limiting aspect of our research.

Investigators should monitor body composition and biological markers (biochemical and hematological) throughout the season to have their own record. However, the lack of blood sampling at the start and after the competition did not allow the possibility of comparing the biological data during the two moments. Each method has its limits, in particular biological markers whose routine availability and reliability are limited to some nutrients.

Changing eating behavior makes it possible to adjust eating practices to the needs of each individual according to gender and age.

In short, our results indicate that athletes rarely do biological examinations. However, the nutritional, hematological and pathological data show a feeding problem. Inadequate or inappropriate nutritional intake is associated with impaired immune function. An insufficiency in protein intake and a rare consumption of adequate foods and particularly vegetables are associated with a lack of iron, therefore with the presence of pathologies such as sports anemia, leukopenia and bicytopenia. These factors most often cause impaired muscle function and limit work capacity, which can lead to a decline in training and athletic performance. They are associated with underperformance, hence the need for appropriate food hygiene and good eating habits for athletes.

5. Conclusion

The interest of this study was to establish a link between diet, hematological parameters and performance in Congolese middle distance and long distance athletes. At the end of this, we can safely say that the nutritional status of Congolese athletes is mostly normal. However, even if the levels of leukocytes, hemoglobin and thrombocytes were significantly good, we noted the presence of abnormalities or nutritional pathologies such as anemia, leukopenia and bycitopenia in the subjects of two groups.

These biological data made it possible to situate the biochemical and hematological status of the athletes. This is why few reliable data are available regarding biological examinations at the start of each sports season and the programming of nutritional education sessions.

Based on our observations, on the nutritional level, we recommended to Congolese athletes:

- Reduce carbohydrate intake in order to maintain a balanced energy balance.
- To increase daily lipid intake by moderately consuming fatty meats (beef, charcuterie) and to increase the consumption of lean meats (chicken, lean part of pork, turkey, etc.) and fish, caterpillars.
- To increase the daily intake of dietary fibers by consuming fruits and vegetables, cereals and breads, nuts (small quantity), beans and peas.
- To balance the consumption of carbohydrates, lipids and proteins in order to respect the standards of dieticians.
- Nutritional status assessment should precede nutritional counseling (including supplementation strategies) and allow its effectiveness to be assessed.
- The evaluation of food intake must be essential, just like that of anthropometric and biochemical characteristics.

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Footnote

BEJ designed and conducted the study and wrote the manuscript. MKPR, NF and MSI participated in data acquisition and performed statistical analyses. The study protocol and the revision of the manuscript were carried out by BAM, MJM, MD, EM, MV, IOYS, NE and MF.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this article.

Informed Consent

This research work received authorization for blood sampling and analysis from the Medical Biology Department of the National Public Health Laboratory of the Republic of Congo, precisely on August 04, 2015 (LNSP-ILP/0122/15. Similarly, the informed consent of each athlete and walker was required after explaining the objectives and interest of the study.

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