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Evaluation of the Anti-oxidant Effet of Spirulina on Marathon Runners in Cote D'ivoire

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Abstract

This experimental intervention research, of the BEFORE-AFTER TYPE was conducted on a sample of ten marathon runners and was undertaken in two sessions of half-marathon separated by a 15-day interval. During this period, each marathon runner took in 5400 mg per day of Spirulina - a microscopic Cyanobacteria-seaweed that is rich in antioxidant substances.

Their oxidative stress was assessed before and after each race with and without Spirulina intake using a spectrofluorometric measurement of the Thiobarbituric Acid Reactive Substances (TBARS), according to the Yagi method as modified by Sess.

The results were as follows:

- Race without Spirulina intake:

TBARS before the race: 1.48 ± 0.631 nmol of malondialdehyde (MDA) per mL of plasma

TBARS after the race: 2.96 ± 0.733 nmol of MDA per mL of plasma

- Race with Spirulina intake:

TBARS before the race: 0.66 ± 0.508 nmol of MDA per mL of plasma

TBARS after the race: 1.46 ± 0.488 nmol of MDA per mL of plasma

We observed that the levels of secondary oxidation were reduced by half with the intake of Spirulina, a reduction that is significant at rest (p=0.027) as well as after the physical effort (p=0.001). At the hemodynamic and anthropometric levels, it was observed that the intake of Spirulina led to a significant increase in weight (65.96 Kg ± 4.16 v 65.49 Kg ± 4.16; p<0.01) and in arterial systolic blood pressure (12.8 mmHg ± 1.13 v 11.4 mmHg ± 0.51; p<0.01).

Our results are supportive of the prescription of Spirulina in most pathological cases, in view of the implication of free radicals in pathogenic life cycles. This would translate into considerable public health benefits.

Keywords: Spirulina; Free radicals; Oxidative stress; Thiobarbituric acid reactants substances (TBARS); Marathon

Introduction

Free radicals are atoms or groups of atoms with at least one unpaired electron; in the body it is usually an oxygen molecule that has lost an electron and will stabilize itself by stealing an electron from a nearby molecule; «in the body, free radicals are high-energy particles that ricochet wildly and damage cells». There are two major groups of radical forms: reactive forms of oxygen free radicals (superoxide $O_2^{\bullet-}$, hydrogen peroxide, hydroxyl radical $HO^{\bullet}$ oxygene singulet) and organic free radicals (Alkoxy radical, peroxyl radical…). Overproduction of free radicals is responsible for a cascade of radical reactions termed oxidative stress [1,2]. They are believed to accelerate the progression of many pathological processes such as cancer, cardiovascular disease, AIDS, hepatitis B, malaria, sickle cell disease, diabetes, major components of public health problems [1-4]. Converging arguments suggest that the production of free radicals may directly or indirectly play an essential role in cellular processes involved in atherosclerosis and carcinogenesis and that an adequate intake of antioxidant nutrients might, (as opposed)[2] opposing to the accumulation of free radicals at the cellular level, play a protective role vis- a-vis cardiovascular disease and cancer [5,6].

The production of free radicals is endogenous (e.g. during transport of electrons in the respiratory chain, in the anti-bacterial defense, during the synthesis of prostaglandins defense and during exercise) and exogenous (UV, alcohol, toxic emissions such as oxide nitrogen, nitrogen dioxide, the tetrachlorure of carbon, antibiotics, anti-cancerous...) [1-3].

The intensive exercise situation promotes the production of reactive oxygen and nitrogen species which exceed the capacity of the body's defense [7,8] and contribute to skeletal muscle fatigue and damage [9]. The main targets of free radicals are: DNA, macromolecules, and lipids. The chemical nature of lipids (cholesterol unsaturated, malonic

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concatenation of polyunsaturated fatty acids, PUFAs, membrane phospholipids) makes them particularly vulnerable to free radicals. Several studies have demonstrated that the level of oxidation after exercise remains high, often persisting for several days after the race [10].

The body has a defense system of endogenous; enzymatic barrier (primarily enzymatic: superoxide dismutase, glutathione peroxidase, catalase) and exogenous the barrier of radical scavengers (from food and consists of antioxidant: flavonoids, carotenoids, vitamin E, vitamin C; but also unsaturated Fatty Acids (UFAs, DNA…). Some of these anti-oxidants are present in large quantities in Spirulina classified among the blue-green algae. It belongs to the class of cyanobacteria with characteristic photosynthetic capability. There are two basic strains: Spirulina maxima and Spirulina platensis [11-13], Spirulina is naturally found in alkaline lakes in equatorial zone. (Mexico, Chad...) [13,14]. Initially known and used for its high protein [13,14], Spirulina is also known for being rich in antioxidants (βcarotene, vitamin E, Phycocyanin and superoxide dismutase) [11,15-17] The antioxydants activities of Spirulina were demonstrated in a large number of preclinical studies. However a limited number of clinical trials have been carried out so far to confirm such activities on humans.

The objective of this study was to evaluate the anti-radical activity of Spirulina by administering to athletes during a marathon race that is to say in a situation of intensive production of Free Radicals.

Methods

Design

The study was an experimental intervention research, conducted ex ante and ex post.

Spirulina used in this study came from the company "SAP DE LA ME", in the area of Adzope in Cote d'Ivoire. West Africa It is the Spirulina platensis strain and is cultivated in controlled conditions. It is harvested, dried, then packaged in powder then in 600 mg Tablets.

Sampling

We chose a team of athletes' marathon of a local company with which the laboratory maintains a working relationship. We selected motivated male athletes who accepted taking Spirulina and have given their informed consent. Athletes taking multivitamin complexes were excluded from the study. A total of 10 athletes were selected.

Ethical permission

We seized the national ethics committee (CNER: Comité National d'Ethique et de Recherche) and obtained a favorable recommendation for the realization of the study.

Framework and duration of the study

Two half marathon races were conducted on the athletics track (400 m) in the Robert Champroux stadium in Marcory (Abidjan) 15 days apart. During this period, each marathon runner took 3 tablets of 600 mg of Spirulina 3 times per day (morning, noon and evening) representing 5.4 g per day.

Biochemical assays (rate of Thiobarbituric Acid Reactant Substances, TBARS, in nmol of MDA / ml of plasma) were performed at the Laboratory of Biochemistry at the Faculty of Medicine of the University Felix Houphouet Boigny in Abidjan.

Anamnestic parameters, clinical-physiological, and parameters related to the sporting activity

Before the first race, information on civil registration particulars, socio demographic characteristics, personal history (related to sport, lifestyle, eating habits), were collected using an interview questionnaire.

Each athlete arrived in a fasting condition, before the first blood sample, the following parameters are measured: weight, temperature, heart rate and blood pressure.

After the race, each athlete was taken care of by first taking pulse and blood pressure, followed by taking the temperature and body weight.

After the measurement of physiological parameters of each athlete, we performed the second blood sample. This chronology was strictly respected for each athlete.

Distance was measured from the number of laps done by each athlete, in the knowledge that one lap is equivalent to 400 meters.

Performance was measured using a stopwatch to time 10 YEMA Type.

Speed was obtained by a simple calculation using the distance (in km) and race time (hours) as inputs.

Blood specimen collection

The Sampling protocol was the same before and after each race (without Spirulina and with Spirulina): 5 ml venous blood in heparinized tube (Lithium Heparin) with each of the subjects having fasted for at least 12 h.

Blood samples were preserved fresh in a cooler containing capacitors and then transported to the laboratory after the second samples taken at the end of the half marathon.

The plasma collected after centrifugation at 4000 rev / min for 30 minutes in a refrigerated centrifuge, was frozen and then 1 ml aliquots at -80°C until analysis.

Determination of degradation products due to oxidative stress

The method used is the spectrofluorometric assay using the YAGI method [18] modified by SESS [19]. It measures substances from lipid peroxidation, TBARS (Thiobarbituric Acid Reactant Substances) reacting with Thiobarbituric Acid (TBA). These include malondialdehyde (MDA), the alkenals, and the alkanals.

Assay principle

It is a method that is based on the principle of hot reaction (95°C) and acidic medium (acetic acid) with two molecules of TBA with each molecule of MDA [20].

This reaction leads to the formation of a complex pink read by fluorometry at the wavelengths (λ): λ excitation of 515 nm and λ 553 nm of issuance. Other aldehydes from lipid peroxidation react in the same way. Staining obtained corresponds to the set of TBA reactive substances (TBARS), but the results are expressed in MDA. It involves two molecules of thiobarbituric acid TBA with one MDA molecule

$$2TBA+1MDA \xrightarrow[\text{Acetic}]{H+} \text{"MDA-TBARS"}_{\text{pink colored complex}}$$

Statistical analysis

Using the ACCESS database, all variables were entered and analyzed using SPSS statistical software. To investigate the normality of the distribution of the different variables, it was used to test of Kolmogorov Smirnov (K-S) specifically dedicated to small sample size.

Regarding quantitative variables descriptive statistical study including calculating averages and standard deviation was performed.

For the comparison of means of the variables before and after the races, it has been used to test the fit BEFORE /AFTER-studies is the paired t-test variables, where, according to the KS test was normal distribution Otherwise, it was used the Wilcoxon test.

The level of significance was 0.05.For the analysis of plasma TBARS levels; we had to remove the subject N°3 because it showed an extreme value which distorted the statistics.

Results

The average age of marathoners was 31.30 years. All male, they had on average 5 years of seniority in the sport.

Physiological parameters without spirulina

Table 1 presents the physiological parameters without Spirulina. During the race without Spirulina, we observed a significant decrease in weight ($p<0.01$) in SBP ($p<0.01$) and DBP ($p<0.05$). An increase was significantly observed in the temperature level ($p<0.01$) and pulse ($p<0.01$).

Physiological parameters with spirulina

Table 2 presents the physiological parameters without Spirulina During the race with Spirulina, we also observed after exercise a significant decrease in weight ($p<0.01$) and DBP ($p<0.05$), a significant increase in temperature ($p<0.01$) and the pulse ($p<0.01$). But the decrease observed at the SBP was not significant.

When comparing physiological parameters in the race with Spirulina and those in the race without Spirulina, there was a significant increase in weight ($p<0.01$), pulse rate ($p<0.05$) and in SBP ($p<0.01$) marathon runners after taking Spirulina.

The performance

Table 3 presents the performances. After taking Spirulina, we observed increasing the distance was not statistically significant.

The plasma TBARS

Table 4 summarize the main results with plasma TBARS. There

Subjects	Weight(Kg)		Temperature (°C)		Pulse (bts/min)		Systolic BP (mmHg)		Diastolic BP(mmHg)	
	Before	After	Before	After	Before	After	Before	After	Before	After
1	63.7	62.1	37.1	39.1	72	132	12	10	8	6
2	64.1	63.3	37.1	38.6	64	132	11	10	8	6
3	66.6	65.0	37.3	37.4	68	132	12	9	8	6
4	60.8	60.1	37.5	37.3	68	132	11	10	7	7
5	61.7	59.7	38.0	38.8	64	118	11	10	8	7
6	68.2	68.2	37.0	37.9	60	108	11	10	7	6
7	62.0	61.2	37.0	37.7	64	128	11	10	7	6
8	73.7	72.7	37.0	38.4	68	92	12	13	8	9
9	62.8	62.0	36.8	37.5	64	116	11	9	8	6
10	71.3	71.3	36.6	38.3	68	124	12	11	8	7
Mean	65.49	64.56	37.14	38.1	66.00	121.40	11.40	10.20	7.70	6.60
SD	4.35	4.64	0.38	0.62	3.40	13.27	0.51	1.13	0.48	0.96

Table 1: Physiological parameters before and after the race without Spirulina.

Subjects	Weight(Kg)		Temperature(°C)		Pulse (bits/min)		Systolic BP (mmHg)		Diastolic BP (mmHg)	
	Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
1	64.5	63.2	36.6	38.6	78	136	15	10	10	8.0
2	64.7	64.4	37.3	39.0	76	134	12	12	7.5	7.0
3	67.0	65.3	36.8	37.5	81	144	12	15	7.5	8.0
4	61.7	59.6	36.4	38.3	68	146	12	10	8.0	5.0
5	62.5	60.6	37.1	39.3	71	168	14	12	9.5	7.0
6	69.2	68.1	36.7	38.5	64	127	12	10	7.0	5.0
7	62.1	61.4	36.8	38.3	71	141	13	12	8.0	7.0
8	73.8	73.0	37.7	37.8	88	116	14	13	9.0	9.0
9	63.1	60.9	36.8	37.8	63	142	12	13	8.0	8.0
10	71.0	70.0	37.3	38.0	104	125	12	12	6.5	6.5
Mean	65.96	64.65	36.95	38.31	76.40	137.9	12.80	11.90	8.10	7.05
SD	4.15	4.46	0.39	0.56	12.39	14.22	1.13	1.59	1.10	1.30

Table 2: Physiological parameters before and after the race with Spirulina.

Subjects	Distance (Km)		Duration		Speed (Km/h)	
	Without Spitulina	With Spirulina	Without Spitulina	With Spirulina	Without Spitulina	With Spirulina
1	21.0	8.2	2h09'09 ''	0h45'57''	9,76	10.71
2	19.0	21.0	2h18'00''	2h23'57''	8.26	8.75
3	14.2	21.0	1h50'00''	2h30'00''	7.75	8.40
4	21.0	21.0	2h22'00''	2h15'55''	8.87	9.27
5	21.0	21.0	2h05'00''	2h02'29''	10.08	10.27
6	18.2	21.0	2h11'00''	2h30'01''	8.34	8.40
7	21.0	21.0	2h31'00''	2h23'57''	8.34	8.75
8	8.2	14.6	1h03'00''	2h02'45''	7.81	7.14
9	21.0	21.0	2h12'00''	2h02'22''	9.55	10.30
10	2.6	12.6	0h46'00''	1h32'54''	3.39	8.14
Mean	16.72	18.24	1h56'43''	2h03'03 ''	8.21	9.01
SD	6.47	4.70	34'44''	32'14''	1.87	1.1229

Table 3: Performances during the race without Spirulina and the race with Spirulina.

Subjects	TBARS(nmol of MDA per mL of plasma)			
	RACE without Spirulina		RACE with Spirulina	
	Before	After	Before	After
1	2.50	3.80	0.78	1.60
2	2.20	3.80	0.50	1.48
4	1.00	1.55	0.78	1.58
5	1.12	2.94	0.50	1.69
6	0.62	3.72	1.10	1.56
7	1.28	2.50	1.73	1.93
8	1.28	2.72	0.20	0.22
9	1.28	2.94	0.20	1.43
10	2.12	2.72	0.18	1.69
Mean	1.48*	2.96**	0.66*	1.46**
SD	0.631	0.733	0.508	0.488
	P=0.000$		P=0.027$	

$ P value means comparison before and after
*Comparison of TBARS before race with and without Spirulina P<0.001
**Comparison of TBARS after race with and without Spirulina P=0.001

Table 4: Plasma TBARS (nmol of MDA/ml of plasma).

was a significant increase in TBARS after the effort, as well without Spirulina (p=0.000) than with Spirulina (p=0.02).

However, we observed a reduction of almost half of the high level of oxidation is taking Spirulina, both at rest (p=0.027; p<0.01)) after exercise (p=0.001).

Discussion

We observed that the levels of secondary oxidation were reduced by half with the intake of Spirulina, as reduction that is significant at rest (p=0.027) as well as after the physical effort (p=0.001).

This result contradicts the results of studies being done in two sessions. According to what is widely described in the literature. It is well established that exercise promotes the production of reactives oxygen and nitrogen species, which contribute to skeletal muscle fatigue and damage [9]. After every marathon there is an overproduction of free radicals resulting in an increase of the degradation products of free radicals often persisting for several days after exercise [8,10]. In the study of 11 marathon runners in Finland, it was observed (by measurement of circulating antioxidants and the oxidizability of LDL) oxidative stress persistence 4 days after the Marathon of the City of Helsinki [10].

The average rate of TBARS after the race with Spirulina is higher than the rate observed before exercise (1.46 ± 0.488 nmol MDA per ml of plasma vs 0.66 ± 0.0508 nmol MDA per ml of plasma; p=0.027), due to the production of secondary free radicals intense physical exercise. However, it should be noted that this rate is lower than that AFTER the race without Spirulina (1.46 ± 0.488 nmol MDA per ml of plasma vs 2.96 ± 0.733 nmol MDA per ml of plasma p=0.001). And, lower than the rate observed before the race without Spirulina (1.46 ± 0.488 nmol MDA per ml of plasma v/s 1.48 ± 0.631 nmol MDA per ml of plasma).

The average plasma TBARS of marathon runners before the race without Spirulina was 1.48 ± 0.63 nmol MDA / ml plasma. It is comparable to the rate observed in the normal Ivorian population (2.1 ± 1.2 nmol / ml) [9] and higher than that observed in the consumer of palm oil (± 1.12 nmol MDA) according to the study by SESS [21,22]. But the average plasma TBARS before the second race(with Spirulina) was significantly lower than the rate observed before the first race (0.66 ± 1.48 nmol MDA vs ± 0.63 nmol MDA, p <0.001) and significantly lower than that observed in populations TBARS levels Lowest, populations consuming crude palm oil (1.12 ± 0.48 nmol MDA / ml plasma).

We observed a significant increase in TBARS after the race (2.96 ± 0.73 nmol MDA / ml vs 1.48 ± 0.63 1 nmol MDA / ml; p=0.000 This significant reduction of the oxidation level can be attributed to the capture of Spirulina. Indeed, after the first race each Marathonien took daily in 3 divided doses for 15 days 5400 mg Spirulina.

Spirulina contains several active ingredients, notably phycocyanin and β carotene that have potent antioxydant and anti-inflammatory activities [21]. The antioxidant power of Spirulina is due to its composition rich in substances scavengers of free radicals (vitamin E, βcarotene, unsaturated lipids...); in antioxidant enzymes such as superoxide dismutase (SOD) catalyzes the dismutation of the superoxide ion. Note also the presence of Spirulina phycocyanin pigment responsible for the specific blue-green Spirulina.

Phycocyanine has the ability to scavenge free radicals, it also decreases nitrite production, and inhibits liver microsomal lipid peroxidation [15,16,22,23].

Our results are similar to those obtained by two clinical trials, conducted to investigate the effect of Spirulina on preventing exercise-induced skeletal muscle fatigue and damage through its antioxidant property. In one study with 16 student volunteers, intake of diet containing 5% Spirulina for 3 weeks resulted a significant reduction of plasma oxidative maker MDA ($p<0.05$) with a current increase in the blood super oxide dismutase activity [24]. In another study with 9 male subjects supplementation of Spirulina with a daily dosis of 8 g for 4 weeks significantly prolonged the time to fatigue, reduced TBARS induced by exercise [25]. Our study is distinguished by the shorter period of administration of Spirulina (2 weeks, 3 weeks vs Lu for study [24] and 4 weeks to study Kalafati [25]) and ingestion topics smaller amounts of Spirulina (5.4 g vs 8 g to study Kalafati). The results obtained in this study allow us to state that taking Spirulina helped to significantly reduce the rate of production of free radicals in the bloodstream, ensuring a significant reduction of oxidative stress.

The role of antioxidant in prevention of chronic diseases is well known. Summarizes the main results of a study, about antioxidant and chronic disease prevention showed after 7.5 years, low-dose antioxidant supplementation lowered total cancer incidence in men [26]. When considering the pathophysiological role of free radicals in the pathogenesis of several diseases, causing problems in public health level, we can say that the administration of Spirulina in the management of these diseases would be a significant contribution. The limitations of this study concerns the actual very reduce population and the use of a single oxidative stress indicator.

Conclusion

Free radicals are believed to accelerate the progression of many pathological processes such as cancer, cardiovascular disease, AIDS, hepatitis B, malaria, sickle cell disease, diabetes, major components of public health problems. Our results argue in favor of the prescription of Spirulina in most pathological cases, in view of the implication of the free radicals in pathogenic life cycles. This would translate into considerable public health benefits.

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