

Effect of *Spirulina* (*Arthrospira platensis*) supplementation on the nutritional proteins in Côte d'Ivoire burn patients

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Abstract: Burn injury as malnutrition remains a major problem throughout the world. Extensive burns contribute to malnutrition with severe body weight loss and negative nitrogen balance. We used *spirulina*, a microscopic blue-green alga is african traditional food which have high content of proteins and several biological activities. So, the aim of this study was to evaluate the effects of *spirulina* on the nutritional proteins (Albumin, total protein, transferrin and Thyroxine-Binding PreAlbumin (TBPA) in the thermal burn. In the 40 subjects, two groups were constituted. The group I (n=20) consists of subjects which receive only the basic daily food ration. The group II (n=20) constituted by subjects receiving the basic daily food ration and the *spirulina* supplementation. Group II: subjects < 5 years=1500mg of *spirulina* (500mgx3 taken), subjects: 5-15years old=300mg of *spirulina*/day (1000mg x3 taken); subjects >15 years=4500mg of *spirulina*/day (1500mg x3taken). Nutritional proteins were measured according to Radial immunodiffusion of Mancini and Biuret method. The results showed increased Blood concentrations of Albumin, Transferrin, TBPA and total Protein in the *spirulina* supplemented. We therefore conclude that, *spirulina* supplementation increase blood concentration of Albumin, transferrin, TBPA and Total protein. The increase blood concentrations of *spirulina* supplemented is due to high content proteins and nutrients that *spirulina* abounds.

Keywords: Nutritional proteins, burn, *spirulina*, Côte d'Ivoire.

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INTRODUCTION

The malnutrition represent a major problem throughout in Africa and was associated with a low synthetic and catabolic rates of proteins;. In other ways, the net transfer of proteins into the intravascular compartment diminished as the catabolic rate fell¹.

Other studies were showed that the malnutrition may result from pancreatic dysfunction and, leading to the hypothesis that the exocrine pancreas is incapable of synthesizing secretory proteins in the absence of dietary protein². It is well known that protein-energy under nutrition, is an energy deficit due to chronic deficiency of all macronutrients³. Malnutrition may be present in hospitalized patients and contributes to increases in both length of stay and hospital costs⁴. Besides, Burn injury remains a major problem throughout the world and affects a large number of individuals each year. Thermal injury induces hypermetabolism of varying intensity and duration depending on the extent and depth of the body surface affected, on the presence of infection and on the efficacy of early treatment. This increased energy expenditure contributes to malnutrition with severe body weight loss and negative nitrogen balance⁵. Also, it was also observed that thermal injury depletes plasma vitamin E⁶, contribute to secondary tissue damage (Oxidative stress) and impaired immune functions⁷.

Supportive interventions to improve the nutritional status of burn patients are an important adjunct to medical therapy⁴. Indeed, the nutrition is not only to provide exogenous nutrients to improve nutritional status of the patients, but also to regulate cell metabolism, enhance cell activity, maintain and uphold the structure and function of the gastrointestinal mucosa, thus to improve patient's outcome³.

According to several studies, *Spirulina* a microscopic blue-green alga is a traditional food for Mexican and African people⁸ because of its high content of proteins as well as essential nutrients like carotenoids, vitamins and minerals. In addition, previous studies have demonstrated its several biological activities such as inhibit viral replication, prevent anemia, decrease genotoxicity induced by drugs, prevent fatty liver disease and has hypoglycemic and hypolipemic properties⁷. It has also been studied its effects on vasomotor responses on aortic rings proposing its antihypertensive activities⁷.

However, the effect of *Spirulina platensis* (*Arthrospira platensis*) on the nutritional proteins for the thermal burn patients has not been evaluated. In this study, we aimed to evaluate the effectiveness of spirulina as supplementation food on blood nutritional proteins (albumin, total protein, transferrin, thyroxine-binding PreAlbumin (TBPA)) in the thermal burn.

SUBJECTS AND METHODS

Patients

The study was designed as a prospective, randomized, case-control trial and was approved by the institutional ethics committee. It was conducted in the burns center of the Service d'Aide Médical d'Urgence (SAMU) of Centre Hospitalier Universitaire (CHU) in Cocody-Abidjan (Côte d'Ivoire), a university hospital. Inclusion criteria were thermal burns involving >50% of burned body surface area (BSA) in patients who were 1 to 25 years old; and written informed consent (from the patients themselves). The study subjects were recruited from April 2006 to January 2007. Finally 40 subjects (22 male, 18 female) were enrolled in this study. All subjects gave written informed consent before beginning the study.

Methods

Spirulina administration

Powdered *Spirulina platensis* (*Arthrospira platensis*) used in the experimental supplementation was marketed by SAP® de la ME (Côte d'Ivoire). During the spirulina supplementation, every 40 burned subjects received the usual therapeutics and the same basic daily food ration (food with carbohydrates, proteins and lipids) of the burn center. The patients were allocated into two groups of 20 persons.

The group I (n=20) consists of subjects which receive only the basic daily food ration. The group II (n=20) constituted by subjects receiving the basic daily food ration and the *Spirulina* supplementation. This group was treated according to the hospital's therapeutic policy.

In the group II, the subjects at least of 5 years old receive a daily dose of 1500mg of *spirulina* (500mg in three taken), those whose age is between 5 and 15years old receive 300mg of *spirulina* per day (1000mg in three taken). This group II was also treated with the same therapeutic treatment as Group I.

Finally, the subject's age which is above 15 years old receive 4500mg of spirulina per day (1500mg in three taken). Every 40 patients were followed during 20 days in the burn center.

Sample collection and preparation

Blood samples were collected from all subjects by test tubes in morning between 7-8 hours at the subjects by venipuncture. Ten millilitres blood sample were taken on admission (zero time or J0) before given *spirulina* supplementation. After the supplementation at J2, J6, J14 and J20, blood sample were also taken each correspondant day to the burns center. Plasma was separated by centrifugation at

3500rpm for 10 minutes at 4 °C and stored at -80°C until nutritional proteins analyzed.

Nutritional proteins determination

Nutritional proteins in particular Albumin, Transferrin, Thyroxine-Binding PreAlbumin (TBPA) were measured according to Radial Immunodiffusion (RID). RID was carried out according to Mancini *et al* (1965) and the immunserums standards used were marketed by Dade Berhing® (Marburg RFA.).Gel of diffusion is agarose type L (strength of gel 1%:850g/cm²; electroendosmose: 0, 10-0, 15) coming from Prolabo® (Fontenay-sous-bois, France)⁹. The Biuret method was used to determine total Proteins.

NPTP or Nutritional Protein Taken Percent=(Daytime Average value -J0Average value)x100/J0Average value.

Statistical analysis

Statistical analysis was performed with the SPSS program (version 12.0). All results are expressed as means±SEM. The global course of each parameter was evaluated by Student and Dunnett (non-parametric) rank tests and then the comparisons between group I and group II. Differences were considered statistically significant when *p* values were less than 0.05.

RESULTS

Generally all biochemical nutritional markers at the *spirulina* supplemented burnt persons or not evolve in the same way to the first (J0) at the second day (J2). The average values of all the biochemical markers fall in a significant way at J2 when they are compared with those of J0 (*p*<0.05). As regards the average values of the Albumin and NPTP between J6 and J20, the average values of J0 are fast obtained at the *spirulina* supplemented subjects than the unsupplemented subjects (Table 1).

Table 1: Spirulina supplementation effects on the blood Albumin concentration value in the thermal burned subjects.

Albumin (g/L)	Days	<i>Spirulina</i> un-supplemented subjects (-)(n=20)		<i>Spirulina</i> supplemented subjects (+)(n=20)	
		Mean ±SD	NPTP(%)	Mean ±SD	NPTP (%)
	J0	28.5±10.01	0	28±8.50	0
	J2	16.5±7 ^{a*}	-72	18±7.01 ^{a*}	-36
	J6	19±7 ^{a*}	-33	19.5±7 ^{a*}	-30
	J14	21±7.5 ^{a*}	-26	23.5±7	-16
	J20	25±11.5	-12	30.5±8	+9

NPTP or Nutritional Protein Taken Percent=(Daytime Average value -J0Average value)x100/J0Average value *p*: significance value ; Means with different superscript letters in the same column are significantly different (*p*<0.05), SD= standard deviation
a*=Low Albumin average value Versus J0 (*p*<0.05)

From J2 to J6, the protein average values grow without affecting J0 values (Table 2). We notice that the total proteins average values increase from J6 to J20 at all subjects. Besides, according to NPTP, total protein is fast obtained for the *spirulina* supplemented (Table 2).

Table 2: *Spirulina* supplementation effects on the blood total protein concentration value in the thermal burned subjects.

Total Protein (g/L)	Days	<i>Spirulina</i> un-supplemented subjects (-) (n=20)		<i>Spirulina</i> supplemented subjects (+) (n=20)	
		Mean ±SD	NPTP (%)	Mean ±SD	NPTP (%)
		J0	57.5±14	0	56.5±11,5
J2	43.5±11 ^{a*}	-24	43.5±7 ^{a*}	-23	
J6	50.5±12,5	-12	52.5±11,5	-7	
J14	60±17	+4	61±24	+8	
J20	67.5±17 ^{b*}	+17	70±17 ^{b*}	+24	

NPTP or Nutritional Protein Taken Percent = (Daytime Average value -J0Average value)×100/J0Average value p: significance value ; Means with different superscript letters in the same column are significantly different (p.<0.05), SD= standard deviation a*=Low Total protein average value Versus J0 (p.<0.05); b *=Increase Total protein Average value Versus J0 (p<0.05) .

We observed in this study with NPTP that the transferrin average value at the spirulina supplemented is fast obtained since J6, although those un-supplemented remained stable on several days (Table 3) (J2- J14).

Table 3: *Spirulina* supplementation effects on the blood Transferrin concentration value in the thermal burned subjects.

Transferrin (g/L)	Days	<i>Spirulina</i> un-supplemented subjects (-) (n=20)		<i>Spirulina</i> supplemented subjects (+)(n=20)	
		Mean ± SD	NPTP(%)	Mean ± SD	NPTP(%)
		J0	1.6±0.4	0	1.6±0.3
J2	1.5±0.2	-6	1.5±0.3	-6	
J6	1.5±0.3	-6	1.6±0.5	0	
J14	1.5±0.3	-6	1.6±0.3	0	
J20	1.6±0.3	0	1.7±0.3	+6	

NPTP or Nutritional Protein Taken Percent =(Daytime Average value -J0Average value)×100/J0Average value

With the TBPA, it was observed according to NPTP that from J6 to J14, the J0 average values are also fast obtained at the supplemented subjects than the un-supplemented (p<0.05).

As for the average values of the un-supplemented subjects, they are later similar to those of J0 (Table 4).

Table 4: *Spirulina* supplementation effects on the blood TBPA concentration value in the thermal burned subjects.

TBPA (mg/L)	Days	<i>Spirulina</i> un-supplemented subjects (-) (n=20)		<i>Spirulina</i> supplemented subjects (+) (n=20)	
		Mean ±SD	NPTP (%)	Mean ±SD	NPTP (%)
		J0	287.5±95	0	260±84
J2	205±57.5 ^{a*}	-29	201±60 ^{a*}	-23	
J6	227±34.5	-21	270±67	+4	
J14	284±100	-1	295.5±87	+14	
J20	323±58.5 ^{b*}	+12	323.5±71.5 ^{b*}	+24	

NPTP or Nutritional Protein Taken Percent=(Daytime Average value -J0Average value)×100/J0Average value p: significance value ; Means with different superscript letters in the same column are significantly different (p.<0.05), SD=standard deviation a*=Low TBPA average value Versus J0 (p.<0.05); b*=Increase TBPA Average value Versus J0 (p<0.05)

DISCUSSION

This study was realized to appreciate the *spirulina* supplementation effects on the nutritional proteins. The study showed that this supplementation was good for the *spirulina* supplemented burnt persons than the un-supplemented subjects. In every case, we observe in the two first days a rough fall of all biochemical parameters protein because the subjects are without food.

This rough reduction is understandable by a protein hypercatabolism and would be associated to burns at the skin level. It is normal that the treatment of the burnt persons is done with a varied food and rich in proteins, carbohydrates, essential lipids, vitamins and trace elements. This food has to allow correcting this metabolic anomaly so that the cure is observed^{10,11}.

The two first days show that all the nutritional proteins concentrations begin to increase and to become later those obtained the first day from burns. What shows that the basic ration proposed in the burn center allows a good care.

The difference between the protein nutritional markers of the two groups (I and II) is observed at the level of their evolution or NPTP. Indeed, the blood total proteins concentration of the *spirulina* supplemented subjects there is above that of the subjects without supplementation from the 6th day. This observation is the same in the days 14 and 20.

At J6, J14 and J20, the various concentrations (albumin, TBPA, transferrin) respective for the *spirulina* supplemented are widely above those without spirulina and those of departure (J0). Here we observe an increase taken significant protein. So, the values of departure (J0) are fast obtained with the *spirulina* supplementation.

With the albumin, the value of J0 is already obtained to J20 at the *spirulina* supplemented what is not the un-supplemented. Transferrin and TBPA, values of departure is obtained to J6 for the supplemented whereas she is respectively obtained to J14 et J20 for the *spirulina* un-supplemented.

All these observations suggest that the *spirulina* supplementation has a beneficial effect in the burnt persons care because she allows restoring quickly the normal protein values than the un-supplemented. This fast increase protein values is due to the composition of the *spirulina*.

Indeed, several studies showed that the *spirulina* would contain high content of proteins, nutrients (carotenoids, vitamins and minerals)^{7,12}. Besides, it was demonstrated that vitamins (Vitamin A)¹³ and the iron¹⁴ participates strongly in the proteins synthesis in particular the Albumin, Transferrin and TBPA what is observed in our study with the *spirulina* supplementation. In addition, *spirulina* has demonstrated its several biological activities such as inhibit viral replication, prevent anemia, prevent fatty liver disease and has hypoglycemic and hypolipemic properties¹⁵⁻¹⁸.

CONCLUSION

This study showed that the blood nutritional proteins at the *spirulina* supplemented subjects normalize very quickly that those have not supplemented because of the high content proteins and nutrients that it abounds.

Although the care of the burnt persons is good with the daily food ration given in the center, it would be desirable to supplement the burn subjects with *spirulina*. This supplementation will obviously allow decreasing the hospitalization daytime what would reduce inevitably the costs of this hospitalization for the burned patients.

REFERENCES

1. Ohishi T, Wang L, Akane H, Shiraki A, Sato A, Uematsu M, Suzuki K, Mitsumori K and Shibutani M. Adolescent hyperactivity of offspring after maternal protein restriction during the second half of gestation and lactation periods in rats. *J. Toxicol. Sci.*, 2012; 37: 345-352.

2. Crozier SJ, D'Alecy LG, Ernst SA, Ginsburg LE and Williams JA. Molecular mechanisms of pancreatic dysfunction induced by protein malnutrition. *Gastroenterology*, 2009; 137: 1093-1101.
3. Arciniegas EL, Ciocchia AM and Hevia P. Effect of the lactose induced diarrhea on macronutrients availability and immune function in well-nourished and undernourished rats. *Arch. Latinoam. Nutr.*, 2000; 50: 48-54.
4. Nguyen GC, Munsell M, Brant SR and LaVeist T. Racial and geographic disparities in the utilization of parenteral nutrition among inflammatory bowel disease in patients diagnosed with malnutrition in the United States. *J. Parenter. Enter. Nutr.*, 2009; 33: 563-568.
5. De-Souza DA and Greene LJ. Pharmacological nutrition after burn injury. *J. Nutr.*, 1998; 128: 797-803.
6. Traber MG, Leonard SW, Traber DL, Traber LD, Gallagher J, Bobe G, Jeschke MG, Finnerty CC and Herndon D. α -tocopherol adipose tissue stores are depleted after burn injury in pediatric patients. *Am. J. Clin. Nutr.*, 2010; 92: 1378-1384.
7. Al-Kaisy AA, Salih Sahib A and Al-Biati HA. Effect of zinc supplement in the prognosis of burn patients in Iraq. *Ann. Burns Fire Disasters*, 2006; XIX: 115-122.
8. Yoshiko I, Fumie S, Junko E, Maiko F and Jian W. Effects of *spirulina*, a blue-green alga, on bone metabolism in ovariectomized rats and hindlimb-unloaded mice. *Biosci. Biotechnol. Biochem.*, 2006; 70: 363-368.
9. Mancini G, Carbonara AO and Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, 1965; 2: 235-254.
10. Torres-Duran PV, Ferreira-Hermosillo A and Juarez-Oropeza MA. Antihyperlipemic and antihypertensive effects of *Spirulina maxima* in an open sample of Mexican population: a preliminary report. *Lipids Health Dis.*, 2007; 6: 33.
11. Ponce-Canchihuamán JC, Pérez-Méndez O, Hernández-Muñoz R, Torres-Durán PV and Juárez-Oropeza MA. Protective effects of *spirulina maxima* on hyperlipidemia and oxidative-stress induced by lead acetate in the liver and kidney. *Lipids Health Dis.*, 2010; 9: 35.
12. Deng R and Chow TJ. Hypolipidemic, antioxidant and anti-inflammatory activities of microalgae *spirulina*. *Cardiovasc. Ther.*, 2010; 28: e33-e45.
13. Yapi Houphouët F, Ahiboh H, Ago K and Monnet D. Profil protéique et Vitamine A chez l'enfant d'âge scolaire en côte d'ivoire. *Ann. Biol. Clin.*, 2005 ; 63: 291-295.
14. Yapi Houphouët F, Ahiboh H, Yayo E, Edjeme A, Attoungbre-Hauhouet ML, Djaman Allico J and Monnet D. Déficit en fer, profil protéique immunitaire, inflammatoire et nutritionnel chez l'enfant de Côte-d'Ivoire. *Cahiers Santé.*, 2009 ; 19: 25-28.
15. Mohamed Ismail F, Doaa Ali A, Augusta F, Mohamed Abdraboh E, Rajiv Gaur L, Wael Ibrahim M, Madhwa Raj HG and Allal O. Chemoprevention of rat liver toxicity and carcinogenesis by *Spirulina*. *Int. J. Biol. Sci.*, 2009; 5: 377-387.

Ohishi T, Wang L, Akane H, Shiraki A, Sato A, Uematsu M, Suzuki K, Mitsumori K, Shibutani M. Adolescent hyperactivity of offspring after maternal protein restriction during the second half of gestation and lactation periods in rats. *J Toxicol Sci.* 37(2):345-52(2012)

2 Stephen J. Crozier, Louis G. D'Alecy, Stephen A. Ernst, Lauren E. Ginsburg, John A. Williams. Molecular mechanisms of pancreatic dysfunction induced by protein malnutrition *Gastroenterology*; 137(3): 1093–1101,(2009).

3 Arciniegas EL, Cioccia AM, Hevia P. Effect of the lactose induced diarrhea on macronutrients availability and immune function in well-nourished and undernourished rats. *Arch. Latinoam. Nutr.*;50(1):48-54,(2000).

4. Nguyen G C, Munsell M., Brant S R, LaVeist T, . Racial and geographic disparities in the utilization of parenteral nutrition among inflammatory bowel disease inpatients diagnosed with malnutrition in the United States *J. Parenter. Enteral. Nutr.*;33(5): 563–568,(2009).

5 Daurea Abadia De-Souza, Lewis Joel Greene. Pharmacological Nutrition After Burn Injury. *J. Nutr.*; 128: 797–803,(1998).

6 Traber MG, Leonard S W, Traber D L, Traber L D, Gallagher J, Bobe G, Jeschke M G, Finnerty C C, Herndon D. a-Tocopherol adipose tissue stores are depleted after burn injury in pediatric patients. *Am. J. Clin. Nutr.*; 92:1378–84,(2010).

7 Al-Kaisy A.A., Salih Sahib A., Al-Biati H.A.H.K. EFFECT OF ZINC SUPPLEMENT IN THE PROGNOSIS OF BURN PATIENTS IN IRAQ. *Ann. Burns. Fire. Disasters*;XIX (3) : 115-122,(2006).

8 Yoshiko I, Fumie S, Junko E , Maiko F, Jian W. Effects of spirulina ,a blue -green alga , on bone metabolism in ovariectomized rats and hindlimb-Unloaded mice. *Biosci. Biotechnol. Biochem.*; 70 (2): 363-368,(2006).

9 Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*;2:235-54,(1965).

10 Torres-Duran P V, Ferreira-Hermosillo A, Juarez-Oropeza M A. Antihyperlipemic and antihypertensive effects of *Spirulina maxima* in an open sample of mexican population: a preliminary report. *Lipids in Health and Disease*;6:33,(2007).

11 Ponce-Canchihuamán J C, Pérez-Méndez O, Hernández-Muñoz R, Torres-Durán P V, Juárez-Oropeza M A. Protective effects of *Spirulina maxima* on hyperlipidemia and oxidative-stress induced by lead acetate in the liver and kidney. *Lipids in Health and Disease*;9:35,(2010).

12 Ruitang Deng, Te-Jin Chow. Hypolipidemic, Antioxidant and Antiinflammatory Activities of Microalgae *Spirulina*. *Cardiovasc. Ther.*; 28(4): e33–e45,(2010).

13 Yapi Houphouët F, Ahiboh H, Ago K, Monnet D. Profil protéique et Vitamine A chez l'enfant d'âge scolaire en côte d'ivoire. *Ann. Biol. clin.*, 63 (3): 291-295,(2005).

14 Yapi Houphouët F, Ahiboh H, Yayo E, Edjeme A, Attoungbre-Hauhouot ML, Djaman Allico J, Monnet D. Déficit en fer, profil protéique immunitaire, inflammatoire et nutritionnel chez l'enfant de Côte-d'Ivoire. *Cahiers Santé.*,19 (1): 25-28,(2009).

15 Mohamed Ismail F, Doaa Ali A, Augusta Fernando, Mohamed Abdraboh E, Rajiv Gaur L, Wael Ibrahim M, Madhwa Raj HG, Allal Ouhtit. Chemoprevention of rat liver toxicity and carcinogenesis by *Spirulina*. *Int. J. Biol. Sci.*, 5(4):377-387,(2009).