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Effects of *Spirulina maxima* on obesity and related diseases

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SUMMARY

In Mexico, Diabetes mellitus, coronary heart disease, stroke, and cirrhosis are leading causes of death among the general population according to the National Institute of Statistics. These diseases are associated with hypertension, obesity, and the metabolic syndrome, sharing insulin resistance. There are many reports suggesting that *Spirulina* (*Arthrospira*) may have a beneficial effect in the prevention of several diseases. In our laboratory, previous studies have demonstrated that *Spirulina* have, among others, hypolipidemic, antihyperglycemic, antihypertensive, and hepatoprotective effects, some of them related with its antioxidant properties. In our perspective, *Spirulina maxima* could be considered as an important complement in prevention and treatment for these disorders.

RESUMEN

En México, la diabetes mellitus, la cardiopatía isquémica, las enfermedades cerebrovasculares y la cirrosis hepática son las causas principales de muerte entre la población general, de acuerdo con el Instituto Nacional de Estadística, Geografía e Informática. Estas patologías están asociadas con la hipertensión, la obesidad y el síndrome metabólico, al compartir la resistencia a la insulina como mecanismo patogénico común. Existen diversos reportes que sugieren que la *Spirulina* (*Arthospira*) puede tener efectos benéficos en la prevención de diversas enfermedades. Los resultados experimentales en nuestro laboratorio han demostrado que la *Spirulina* tiene propiedades hipolipidémicas, antihiperglicémicas, antihipertensivas, y hepatoprotectoras, algunas de ellas relacionadas con su efecto antioxidante. En nuestra perspectiva, la *Spirulina maxima* podría ser considerada como complemento en la prevención y tratamiento de pacientes con estas enfermedades.

ABBREVIATIONS

AMPK	AMP-dependent protein Kinase
BMI	Body Mass Index
Carbachol	Carbamoylcholine
CCl_4	Carbon tetrachloride
DIAST-P	Diastolic blood pressure
FFA	Free Fatty Acids
HDL	High Density Lipoproteins
HDLc	High Density Lipoprotein- cholesterol
IL-6	Interleukin 6
Indom	Indomethacin
JNC	Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure
LDL	Low Density Lipoproteins
LDLc	Low Density Lipoprotein- cholesterol
L-NAME	Nω-nitro-L-arginine methyl ester hydrochloride
NAFLD	Non-alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steato Hepatitis
NO	Nitric Oxide
PD_2	-log of mean molar concentration causing 50% of maximal response
SFA	Saturated Fatty Acids
SREBP-1c	Sterol Regulatory Element Binding Protein 1-c
SYST-P	Systolic Blood Pressure
TAG	Triacylglycerols
TBARS	Thiobarbituric Acid-reactive Substances
TNFα	Tumor Necrosis Factor α
UFA	Unsaturated Fatty Acids
VLDL	Very Low Density Lipoproteins

INTRODUCTION

Clinically, in adults obesity is considered when BMI (body mass index) > 30 kg/m^2; however, some pathophysiologic changes could be occurring in subjects with overweight (BMI > 25 kg/m^2). The mean BMI at diagnosis of diabetes is 28 kg/m^2,[1,2]. Obesity is a condition of excess fat accumulation that leads to multiple pathological consequences such as metabolic

diseases like diabetes, hyperlipidemia, non-alcoholic fatty liver, and hypertension. Obesity is a risk factor for diabetes, myocardial infarction, stroke, among other ones.[1,2]

The studies here reviewed summarize results regarding the effects of *Spirulina* on: a) plasma and liver lipids of experimental non-alcoholic fatty liver disease (NAFLD) in Wistar rats; b) on the vasomotor reactivity of aortic rings excised from either lean or obese Wistar rats; c) finally, the effects of *Spirulina* intake on prevalences of hypertension and dyslipidemias in human volunteers.

ADIPOSE TISSUE AS AN ENDOCRINE SYSTEM

It is well known that adipose tissue is not a simply lipid storage. Instead it secretes a high number of vasoactive factors, including vasoconstrictor prostanoids, angiotensin II, prostaglandins, and nitric oxide (NO).[3,4] Another group of active molecules, tissue-specific, called adipocytokines, could be related with insulin resistance or altered insulin sensitivity, and in fact some of them have been associated with suppressed hepatic insulin sensitivity and in development of liver fibrosis.[5]

Adiponectin. Some studies have shown reduced plasma levels of this adipocyte secreted protein in obesity, insulin resistance, type 2 diabetes, and in Non-alcoholic Fatty Liver Disease. Adiponectin has antilipogenic effects, stimulates mitochondrial β-oxidation by activating AMP-dependent protein kinase (AMPK) and decreases synthesis of sterol regulatory element binding protein 1-c (SREBP-1c), reducing malonyl-CoA levels and increase fatty acid oxidation, leading to reduced liver triacylglycerols (TAG) accumulation. It may antagonize the effects of inflammatory mediators as tumor necrosis factor α (TNFα) and attenuate the progression of NAFLD by reducing hepatic stellate cell proliferation and increases apoptosis.[6,7]

Leptin. Participates in body weight regulation, modulating the appetite sense, acts on hypothalamic center, and contributes in adipose tissue development. In addition, may contribute to increased sympathetic nervous system activity that is typically found in obesity, it may enhance sodium retention and volume expansion, both key features in the pathophysiology of obesity-associated hypertension.[3] It has been demonstrated that leptin failure action is the major mechanism for leading to hepatic TAG accumulation and hepatic steatosis.[8]

Resistin. Is induced during adipogenesis and secreted by adipocytes. It has been probed an increase levels in NAFLD patients with a good correlation to severity of the disease.[9]

TNFα. Adipose tissue is the major source of this cytokine and has many functions such as antagonizing the effects of adiponectin and contributing to insulin resistance, with impairment on mitochondrial function.[10]

OBESITY AND NON-ALCOHOLIC FATTY LIVER DISEASES

Non alcoholic liver disease shares the mechanism of insulin resistance with the metabolic syndrome (obesity, diabetes, hypertension, dyslipidemia). Nevertheless, some studies

have shown that fatty liver could be the source of insulin resistance and metabolic syndrome in obese people because insulin requirements in subjects with Diabetes mellitus type 2, correlate with the severity of steatosis. Hepatic insulin resistance is present in many patients with NAFLD.[11-13] This resistance could be induced by the free fatty-acid from hepatocyte inflammation, with the subsequent release of systemic proinflammatory cytokines including TNFα and IL-6.[5]

Patients with steatosis (12-30%) progress to NASH and mild fibrosis, 12-20% of those with NASH will progress to advanced fibrosis (scarring) and 12-35% of patients with advanced scarring will develop cirrhosis.[5,14]

Reactive species are involved in NAFLD pathophysiology, according to "two hits theory". When liver is exposed to first hit there are increases of fatty acid flux to mitochondria. These lipids are vulnerable to oxidative stress, increasing consequently cytokines involved in fibrogenesis release (second hit).[15,16]

OBESITY AND CARDIOVASCULAR DISEASES

Cardiovascular diseases, specifically hypertension, myocardial infarction and stroke, are leading causes of morbidity and mortality in developed countries.[17] Classical risk factors for these diseases include, obesity, sedentary lifestyle, smoking, dyslipidemias, post menopausal estrogen deficiency, type II diabetes mellitus, and atherosclerosis.[18-21] In recent years, it has been recognized that atherosclerosis is an inflammatory disease,[22] and, hence, elevated serum levels of inflammatory markers (like C-reactive protein) are now considered both risk factors and predictors of future cardiovascular events.[23] All of the above-mentioned risk factors are associated with an altered vascular reactivity, characterized by an increased responsiveness to vasoconstrictor stimuli and decreased vasodilator responses. This altered vascular reactivity is caused, at least in part, by endothelial dysfunction, mainly decreased release of nitric oxide and vasodilating prostanoids[24-26] and an increased release of endothelin and vasoconstricting prostanoides.[4,27-33]

EFFECTS OF SPIRULINA MAXIMA

Spirulina (*Arthrospira*) is a group of blue-green cyanobacteria. For centuries, *Spirulina* has been a traditional food in some cultures, more recently it is used as a nutritional supplement throughout the world, and there are several reports suggesting that *Spirulina* may have a beneficial effects as antiviral, immunomodulator, antineoplasic, antioxidant, hypolipidemic, antihyperglycemic, antihypertensive, and hepatoprotective effects.[34-41]

Emerging evidence has shown that, both in humans and in animal models, the intake, as a food supplement, of the non toxic non teratogenic blue-green cyanobacteria *Spirulina* has beneficial effects on most of the above mentioned risk factors.[42-45] Such effects have been related to one or more of the biochemical constituents of *Spirulina* which include phycocyanin (an cyclooxygenase type II inhibitor and antioxidant),[46-49] gamma linolenic acid (an essential fatty acid and precursor of arachidonic acid),[45] carotenes, tocopherol[50] as well as Na- and Ca-Spirulan.[51,52]

These risk factors are associated with an altered vascular reactivity, characterized by an increased responsiveness to vasoconstrictor stimuli and decreased vasodilator responses. This altered vascular reactivity is caused, at least in part, by endothelial dysfunction, namely a decreased release of endothelium derived vasodilator mediators (mainly nitric oxide and vasodilating prostanoids)[24-26] and an increased release of vasoconstrictor agonists (mainly endothelin and vasoconstricting prostanoids).[4,27-33]

OUR CONTRIBUTIONS

1. Plasma and liver lipids of experimental non-alcoholic fatty liver disease in Wistar rats

Studies in our laboratory have demonstrated the hepatoprotective effects of *Spirulina maxima* against damage produced by carbon tetrachloride (CCl_4),[38,39,53] a hepatotoxin that induces steatohepatitis as NAFLD model.

After CCl_4 treatment, liver total lipids, TAG and final products of lipoperoxidation were significantly lower in rats fed on a diet with 5% *Spirulina* than in rats without *Spirulina* supplement ($p < 0.05$, table 11-1). Similar findings were observed when defatted and oil fractions were assessed (data not shown). Furthermore, after CCl_4 treatment the values of the liver microsomal thiobarbituric acid-reactive substances (TBARS) were lower in the *Spirulina* group than in the control group, In addition, when the rats were fed on a *Spirulina* diet and treated only with the vehicle, the percentage of HDL levels were increased in comparison to control diet group. CCl_4 treatment induced increments on VLDL and LDL percentages, but these changes were not observed in *Spirulina* group (data not shown).[53]

The results above described show the preventive effect of dietary *Spirulina* on serum and liver lipid changes induced by CCl_4. Also, they are suggestive that antioxidant effect of *Spirulina* could be involved in the hepatoprotection.

On the other hand, when fatty liver was induced by a sublethal dose of CCl_4 in order to analyze the early effects of this hepatotoxin on free fatty acid profile, it was found that

Table 11-1. Effects of Spirulina on liver lipids from rats treated with CCl_4

Treatment	Diet group	Total Lipids (mg/g liver)	Total TAG (mg/g liver)	Microsomal TBARS (µg/g)
	Control	38.7 ± 2.6	0.83 ± 0.8	12.3 ± 3.31
Vehicle	Spirulina	38.4 ± 4.7	1.65 ± 0.5	12.1 ± 1.99
	Control	50.0 ± 4.6 *	8.87 ± 1.7*	14.2 ± 0.10*
With CCl_4	Spirulina	41.5 ± 5.5	1.82 ± 0.7	10.9 ± 1.40

Animals were fed on purified diets, control or experimental 5% Spirulina. Treated with a single dose of either vehicle or CCl_4 (1 mL/kg). After 96 h post-treatment, the rats were killed and the liver excised. TAG, triacylglycerols; TBARS, thiobarbituric acid reactive substances. Values are expressed as mean ± SD per g wet liver of n = 6. *Significantly different from all groups ($p < 0.05$, Multiple comparison Tuckey test). Data from references.[38,53]

CCl_4 increase the content of liver free fatty acids in both groups (table 11-2); however, this increase was attenuated in animals fed on *Spirulina* diet. In addition, CCl_4 treatment induces an increment of liver content of unsaturated fatty acids, but as mentioned before, this change was attenuated by *Spirulina* diet. The results suggest that, in the fatty liver induced by CCl_4, the hepatoprotective effect of *Spirulina* involves: a) an antioxidant mechanism and b) a lower unsaturation of the liver fatty acids. The preventive effect of *A. maxima* on the liver lipid changes induced by CCl_4 could be partially explained by its antioxidant action and the ability to increase the synthesis/release of nitric oxide.

2. Spirulina and vasomotor reactivity

Based in above mentioned antecedents, the studies here reviewed were designed to explore possible effects of *Spirulina* on the vascular reactivity of aortic rings excised from either lean or obese Wistar rats, using an experimental model that allows to identify with certainty if a given vascular response is or not endothelium-dependent. In these studies only two vasoactive agonists were tested. Vasoconstriction was elicited by the α_1-adrenoceptor agonist phenylephrine while vasodilation was explored with carbachol (carbamoylcholine), an endothelium-dependent muscarinic agonist. To identify nitric oxide mediated vasodilation L-NAME was used to inhibit nitric oxide synthesis. To identify responses mediated by arachidonic acid derived, cyclooxygenase-dependent prostanoids, the cyclooxygenase inhibitor indomethacin was used.

In these series of experiments the effects of a raw ethanolic extract of *Spirulina* (water/ethanol, 9:1, v/v), on the vasomotor responses of aortic rings excised from lean or obese rats were analyzed.

Effects of the ethanolic extract of Spirulina maxima on endothelium dependent vasomotor responses of rat aortic rings

In the presence of the *Spirulina* extract aortic rings with endothelium developed less tension in response to phenylephrine than in its absence. This effect was concentration-dependent (0.06–1.0 mg/mL; lyophilized extract dissolved in the bathing solution) (figure 11-1).

Table 11- 2. Effects of Spirulina on liver lipids from rats treated with CCl_4

Treatment	Diet group	Total Lipids (mg/g)	Total TBARS (µg/g)	Total FFA (µg/g)	SFA (%)	UFA (%)
Vehicle	Control	45± 6	1.8 ± 0.4	44 ± 26	93 ± 18	27 ± 17
	Spirulina	48 ±5	2.0 ± 0.4	61 ±43	89 ± 26	13 ± 18
With CCl_4	Control	70 ±17	5.4 ± 1.9*	537± 251[a]	67 ± 14	48 ± 24
	Spirulina	67 ±3	2.6 ± 0.7	214 ± 56[a]	76 ± 18	26 ± 7

Animals were fed on purified diets, control or experimental 5% *Spirulina*. Treated with a single dose of either vehicle or CCl_4 (2 mL/kg). After 48 h post-treatment, the rats were killed and the liver excised. TBARS, thiobarbituric acid reactive substances; FFA, free fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids. Values are expressed as mean ± SD per g wet liver of n = 6. *Significantly different from all groups ($p < 0.05$, Multiple comparison Tuckey test). [a]Significantly different from vehicle control groups and between CCl_4 treated groups. ($p < 0.05$, Multiple comparison Tuckey test). Data from reference.[39]

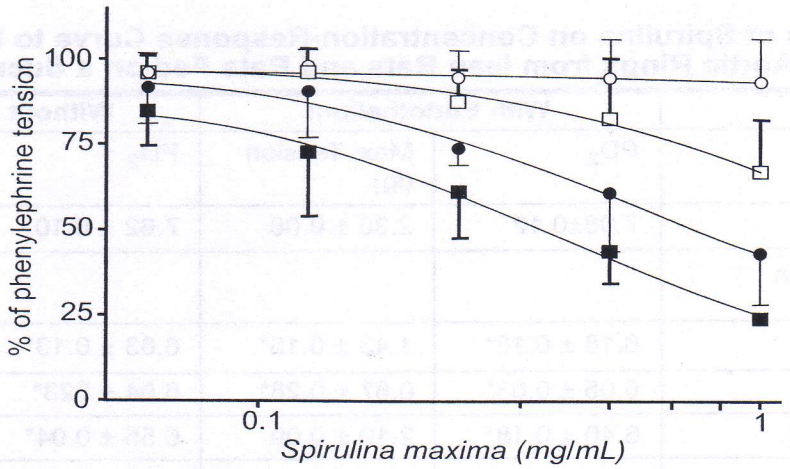

Figure 11-1. Relaxant effects of *Spirulina maxima* extract on aortic rings from lean rats (circles) and obese rats fed on a sucrose rich diet (squares), precontracted with phenylephrine (10^{-5} M). The figure illustrates the cumulative concentration-response curves to *Spirulina maxima* extract in rings with (filled) and without endothelium (open). Data are expressed as percent of maximal phenylephrine-induced tension and shown as means ± SD of five rats.

The attenuation of the response to phenylephrine decreased after inhibiting the cyclooxygenase with indomethacin, the decrease was, however not significant. The inhibition of the nitric oxide synthetase with L-NAME reverted the effects of the *Spirulina* extract (table 11-3).

These results suggest that the extract either increases the synthesis/release of nitric oxide by the endothelium or the biological action of NO on vascular smooth muscle cells.

In endothelium-denuded rings, the addition of indomethacin in the presence of the extract decreased maximal phenylephrine-induced tension to less than 50% of that observed before inhibiting the cyclooxygenase (table 11-3). This indicates that, in the presence of the extract, at least 50% of the tension developed by endothelium-denuded rings in response to phenylephrine is elicited by a cyclooxygenase dependent vasoconstrictor prostanoid. Since on rings with an intact endothelium, the addition of indomethacin in the presence of the extract did not decrease significantly maximal tension development, such a vasoconstrictor prostanoid must have its origin in non-endothelial cells; possible candidates are either vascular smooth muscle cells or fibroblasts of the adventitia. It is quite plausible, that the high content of gamma linolenic acid of *Spirulina*, a precursor of arachidonic acid, plays a role in the synthesis of such a vasoconstrictor prostanoid.

In phenylephrine precontracted aortic rings, the addition of cumulatively increasing concentrations of *Spirulina* extract to the superfusing solution produced a relaxant effect. This effect was both concentration- and endothelium-dependent (figure 11-1).

The concentration-response curve to carbachol (10^{-9} - 10^{-5} M) of rings with endothelium; was not modified significantly by the addition of the *Spirulina* extract (0.5 mg/mL). The lack of effect of the extract on the carbachol induced relaxation of phenylephrine-precontracted rings, indicates that the extract does not enhance the biological activity of nitric oxide or protect its breakdown, and, also, that the extract increases the tone related synthesis/release of NO but not the receptor-mediated release.

Table 11-3. Effects of Spirulina on Concentration-Response Curve to Phenylephrine (10^{-9} – 10^{-5} M) on Aortic Rings from lean Rats and Rats Fed on a Sucrose Rich Diet

Group	With Endothelium		Without Endothelium	
	PD_2	Max. Tension (ig)	PD_2	Max. Tension (g)
Normal diet (A)	7.08±0.12	2.36 ± 0.08	7.62 ± 0.10	2.20 ± 0.19
Normal diet pus Spirulina Extract				
(-) indom.	6.18 ± 0.18*	1.43 ± 0.15*	6.63 ± 0.13*	2.43 ± 0.18
(+) indom.	6.05 ± 0.03*	0.67 ± 0.28*	6.54 ± 023*	0.93 ± 0.21*
(+) indom. plus L-NAME	6.40 ± 0.18*	2.10 ± 0.09	6.55 ± 0.04*	2.28 ± 0.09
Sucrose diet (B)	6.94 ± 0.04	2.65 ± 0.64	7.41 ± 0.19A	2.15 ± 0.48
Sucrose diet plus Spirulina extract				
(-) indom.	6.76 ± 0.11*A	0.97 ± 0.44*	6.95 ± 0.09*	2.27 ± 0.35
(+) indom.	6.10 ± 0.02*	0.45 ± 0.24*	6.38 ± 0.12*	1.72 ± 0.58
(+) indom. plus L-NAME	6.59 ± 0.08*	2.45 ± 0.39	6.70 ± 0.08*A	2.57 ± 0.45

PD_2, -log of mean molar concentration causing 50% of maximal response to phenylephrine (10^{-5} M); Max. tension, maximal tension (g) developed in response to phenylephrine (10^{-5} M); (+) indom, with indomethacin (10^{-6} M); (+) L-NAME, with L-NAME (300 μM).
Data are presented as mean ± SE (PD_2) or mean ± SD (Max. tension); n = 5 for all experimental conditions. *Denotes a significant difference with the control group A or B ($p < 0.05$). Values marked with A are significantly different from the corresponding values of the group A ($p < 0.05$).

Effects of the ethanolic extract of Spirulina maxima on the vasomotor responses of aortic rings from obese sucrose-fed rats

Rats used for these experiments were fed on a sucrose-rich diet during six weeks. After that treatment they were significantly overweight, had elevated blood pressure and hyperlipidemia. According to existing reports using this model of the metabolic syndrome[22,33-36] such rats are also hyperinsulinemic and insulin resistant. Hypertension in metabolic syndrome has been attributed to multiple simultaneous factors. These factors lead ultimately to increased vascular resistance, either by an increase in vasoconstrictor activity or a decrease in vasodilator responses.

Aortic rings with endothelium excised from obese rats developed more tension in response to phenylephrine than those from lean rats; however, the difference was not significant. Phenylephrine precontracted rings, relaxed less in response to carbachol than corresponding rings from lean rats. Similarly, the *Spirulina* extract produced in aortic rings from obese rats (like in aortic rings from lean rats) a concentration-dependent decrease of the contractile response to phenylephrine. The reduction of the contractile response was much larger in rings having an intact endothelium than in endothelium-denuded rings (figure 11-2).

In the presence of both the extract and indomethacin, tension induced by phenylephrine was less than that recorded when only the *Spirulina* extract was present; however the difference was not significant. When the concentration-response curve to phenylephrine was performed in the presence of the extract, after inhibiting the nitric oxide

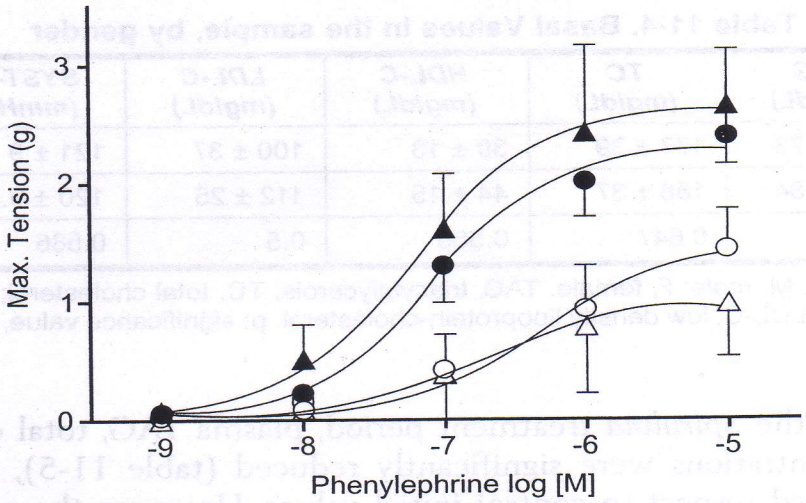

Figure 11-2. Effects of Spirulina maxima extract on the concentration–response curves to phenylephrine (10^{-9} – 10^{-5} M) on aortic rings with endothelium from lean rats (circles) and obese rats fed on a sucrose rich diet (triangles). The curves were obtained in the absence of Spirulina maxima extract (filled), or in the presence of Spirulina maxima (500 mg/mL) (open). Data are presented as means ± SD of five rats.

synthase with L-NAME, phenylephrine-induced tension was similar to that induced in the absence of both the extract and indomethacin. As in the aortic rings from lean rats, the *Spirulina* extract caused a concentration-dependent relaxation of phenylephrine-precontracted rings; however, in rings from obese rats the effect of the extract on phenylephrine-induced tension was strictly endothelium dependent and much greater than in rings excised from lean rats (figure 11-1). Mean reduction of maximal tension by the *Spirulina* extract was approximately 65% in rings from obese rats and 40% in rings from lean rats.

As in rings from lean rats the concentration-response curve to carbachol of rings with endothelium from the obese rats was not modified significantly by the addition of the *Spirulina* extract, indicating that the extract increases only the tone related synthesis/release of NO.

3. Effects of Spirulina intake on prevalences of hypertension and dyslipidemia in human volunteers

The purpose of this study was to evaluate the effects of *Spirulina maxima*, orally administered, on plasma lipids and blood pressure in a Mexican population after six weeks of treatment, as a possible alternative treatment for dyslipidemia and hypertension.[40]

As shown in table 11-4, the initial lipid values were not different between male and female subjects, suggesting a possible integration as a single group. The initial triacylglycerol concentrations were 254 ± 173 and 217 ± 184 mg/dL, for male and female groups, respectively, this finding suggested a hypertriacilglycerolemic tendency in the analyzed sample. The other lipid levels were in optimal or limited ranges. In addition, at the beginning of the study both, systolic or diastolic blood pressures were not different between male and female volunteers.

Table 11-4. Basal Values in the sample, by gender

	TAG (mg/dL)	*TC (mg/dL)*	*HDL-C (mg/dL)*	*LDL-C (mg/dL)*	*SYST- P (mmHg)*	*DIAST-P (mmHg)*
M(n = 16)	254 ± 173	177 ± 39	39 ± 13	100 ± 37	121 ± 9	85 ± 6.5
F(n = 20)	217 ± 184	186 ± 37	44 ± 15	112 ± 25	120 ± 9.5	85 ± 11
p	0.89	0.647	0.588	0.5	0.586	0.185

Values are mean ± SD. M, male; F, female. TAG, triacylglycerols; TC, total cholesterol; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol. p: significance value, Student's-t test. Data from reference.[40]

At the end of the *Spirulina* treatment period, plasma TAG, total cholesterol (TC), and LDL-C concentrations were significantly reduced (table 11-5), whereas HDL-C levels were increased respect to control initial values. However, the univariate analysis showed that the changes on HDL-C and TC concentrations were dependent on TAG concentration; whereas LDL-C concentration was independent of TAG values (p = 0.044).

Respect on blood pressure (table 11-5), there were significant differences either between initial and final systolic and diastolic blood pressure. Furthermore, a significant decrease on systolic blood pressure was observed since the fourth week of *Spirulina* consumption. Dyslipidemias prevalence's are shown in table 11-6. Hypertriacylglycerolemia in the sample was 41.7% *vs.* a final prevalence of 22.2%. Furthermore, significant differences were found between male and female groups (initial hypertriacylglycerolemia prevalence 62.5% *vs.* 25.8%, p = 0.026; final prevalence 43.8% *vs.* 5%, p = 0.008, respectively, results not shown). The initial hypercholesterolemia prevalence (TC ≥ 200 mg/dL) was of 27.8%, but it was diminished after the treatment to 13.9%. The most important changes were observed if the higher values of cholesterol (TC ≥ 240 mg/dL) were used

Table 11-5. Effects of Spirulina treatment on plasma lipid concentrations and blood pressure

Variable	TAG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	SYST-P (mm Hg)	DIAST-P (mm Hg)
Initial	234 ± 178	182 ± 37	43 ± 14	103 ± 30	120 ± 9	85 ± 9
Final	168 ± 101	163 ± 34	50 ± 19	86 ± 28	109 ± 9	79 ± 8
p	0.001a	0.001a	0.01a	0.013a	< 0.001b	<0.05b
Univar. p		0.108	0.247	0.044		

Values are mean ± SD; n = 36; [a] Student's t test or [b] Tuckey's multiple comparison test; Univar. p = univariated p. p: significance value. Data from reference.[40]

Table 11-6. Dyslipidemia prevalence in the sample (cases)

Variable	TAG* (mg/dL) >200	TC* (mg/dL) >200	HDL-C* (mg/dL) >35	LDL-C* (mg/dL) >130
Initial (n = 36)	15	10	26	9
Final (n = 36)	8	5	28	3

* p <0.05 initial vs. final, chi-squared test. Data from reference.[40]

Table 11-7. Initial and Final High Blood Pressure Prevalences* (cases)

BP	Normal	Prehypertension	Hypertension Stage 1	Hypertension Stage 2
Initial (n=36)	4	16	11	5
Final (n=36)	13	18	4	1

BP: blood pressure. * p = 0.01 initial vs. final, chi-squared test, n = 36. Data from reference.[40]

for the analysis of hypercholesterolemia, the initial prevalence was 8.3%; and after the *Spirulina* treatment was 0.0%. Initial hypoalphalipoproteinemia prevalence was 27.7% (10/36, cases); and at the end of treatment, prevalence was only 22.0% (8/36 cases).

According JNC 7 blood pressure reference values, high blood pressure prevalence was assessed in total sample before and after treatment with *Spirulina* (table 11-7). The results show that the initial Hypertension type 2 (>160/ >100 mm Hg) prevalence was 14%, but it was diminished after the treatment to 3% whereas Hypertension type 1 (140-159/90-99 mm Hg) prevalence diminished from 31% to 11%. Furthermore, an increase on pre-hypertension (120-139/ 80-89 mm Hg) prevalence from 44% to 50%, and normal blood pressure prevalence from 11% to 36% were found.

CONCLUSIONS AND PERSPECTIVES

In this study the first step of two hits theory for NAFLD development was analyzed, demonstrating that dietary *Spirulina* attenuated the effects of hepatic oxidative stress induced by CCl_4 treatment. Further studies will analyze the subsequent stages in NAFLD development.

In regard of *Spirulina* effects on vasomotor reactivity the results strongly suggest that the *Spirulina* extract induces a tone-related increase in the synthesis/release of NO by the endothelium. An increased bioavailability of NO, in the presence of the extract, seem less likely since the extract had no effect on the carbachol-induced relaxation of rings with a functional endothelium. The results obtained in these studies allow, also conclude that the extract increases in aortic rings from lean rats the synthesis/release of a cyclooxygenase-dependent vasoconstrictor prostanoid by non-endothelial cells. In rings from obese rats, the extract, in addition to increasing the synthesis/release of NO, also, seems to inhibit the synthesis/release of a cyclooxygenase-dependent vasoconstrictor metabolite of arachidonic acid, which is known to be increased in obesity.

In humans, *Spirulina maxima* intake decreases blood pressure and plasma lipid concentrations, especially TAG and LDL-C, and indirectly modifies the TC and HDL-C concentrations.

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