

# The hepatoprotective and hypolipidemic effects of *Spirulina* (*Arthrospira platensis*) supplementation in a Cretan population with non-alcoholic fatty liver disease: a prospective pilot study

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## Abstract

**Background** A pilot study was conducted to determine the effects of *Spirulina* (*Arthrospira platensis*) on Cretan patients with non-alcoholic fatty liver disease (NAFLD). *Spirulina* is a filamentous cyanobacterium taken as a dietary supplement.

**Methods** Fifteen adult Cretan outpatients (13 men), median age 48 (range: 29-62) years, with NAFLD were orally supplemented with 6 g of *Spirulina* (Greek production) per day for six months. Anthropometric characteristics (height, weight, waist circumference), systolic and diastolic blood pressure, complete blood count, biochemical assessments, homeostasis model assessment of insulin resistance (HOMA-IR) index, health-related quality of life and abdominal sonographic findings were recorded and measured, before and after *Spirulina* supplementation.

**Results** At the end of the 6-month intervention period, the mean levels of aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, triglycerides, low-density lipoprotein-cholesterol, total cholesterol, and the ratio of total cholesterol to high-density lipoprotein cholesterol were significantly decreased: 38.5%, 37.5%, 26.7%, 24.8%, 9.6%, 9.1%, and 13.5% respectively, whereas the mean levels of high-density lipoprotein-cholesterol and hemoglobin were significantly increased: 4.2% and 4.1% respectively. *Spirulina* supplementation resulted also in a significant reduction in weight and HOMA-IR index (8.1% and 19.6% respectively) and a significant improvement in health-related quality of life scale. No changes in sonographic findings were observed.

**Conclusion** *Spirulina* supplementation at a high dosage of 6 g daily in NAFLD patients has strong and multiple beneficial metabolic effects and improves their health-related quality of life.

**Keywords** *Arthrospira*, lipids, liver, NAFLD, spirulina, steatosis

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## Introduction

*Spirulina*, a blue-green alga, is a microscopic and filamentous cyanobacterium that has been used for centuries as a dietary supplement for both humans and animals. The two primary producers of *Spirulina* are two species of cyanobacterium,

classified in the Genus *Arthrospira* as *Arthrospira platensis* (*A. platensis*) and *Arthrospira maxima* (*A. maxima*) [1,2]. It is listed at present as a safe food supplement by the US Food and Drug Administration (category: Generally recognized as safe), nutraceutical, due to its high nutritional value (rich source of proteins, vitamins, minerals, carotenoids, and phycocyanins) and its proven safety in many toxicological studies [1-4]. Some particular species of *Spirulina* have also exhibited metabolic (hypolipidemic, hypoglycemic), anti-viral, liver-protecting and blood-vessel relaxing effects, anti-cancer, anti-inflammatory and anti-oxidant properties, in addition to positive effects on both innate and specific immunity [1,4].

Non-alcoholic fatty liver disease (NAFLD) is a common liver pathology characterized by lipid accumulation in the liver and affects up to 30% of the Western population [5,6]. NAFLD, regarded as the hepatic manifestation of the metabolic syndrome (MetS), is a cluster of metabolic abnormalities associated with insulin resistance (IR), including central obesity and dyslipidemia [5]. Clinicopathologically, it includes a broad

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spectrum of histologic abnormalities and clinical outcomes, ranging from benign hepatic steatosis to cirrhosis, liver failure or hepatocellular carcinoma [5-11]. Pathophysiologically, accumulating data indicate that IR, mitochondrial dysfunction, oxidative stress, lipotoxicity and altered redox balance have a key role in the pathogenesis of steatosis, steatohepatitis (NASH), and fibrosis [5-11]. For diagnosis and prognosis of NAFLD, liver biopsy is the gold standard. Simple steatosis is considered as a benign state, while NASH, because of elevated liver enzymes [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)], histologic (steatosis with perilobular inflammation or hepatocyte ballooning, Mallory hyaline and acidophil bodies with or without fibrosis) and coexisting metabolic (such as diabetes mellitus, obesity, dyslipidemia, hypertension) abnormalities, is regarded as a severe condition [6,8-10]. Moreover, coronary artery disease is the most common cause of death in patients with NAFLD [11-13]. The necessity for an effective therapy is both explicit and urgent. Although recent therapies such as pioglitazone, vitamin E, omega 3 polyunsaturated fatty acids and statins have been shown to be beneficial for the management of NAFLD, more research is needed to assess the impact of these treatments on a long-term basis [6]. Because of the absence of proven treatments, the cornerstone therapy of NAFLD is directed toward weight loss, lifestyle modifications, and comorbidity management [6,9,11].

The aim of this pilot study was to ascertain the effects of orally supplemented *Spirulina (A. platensis)* on Cretan patients with NAFLD and to demonstrate the efficacy of *Spirulina* as a potential alternative therapy for NAFLD.

## Patients and methods

### Subjects

Adult outpatients of both genders (n=15; 13 men) with NAFLD attending the Outpatient Clinic of Internal Medicine of the Naval Hospital of Crete participated in this prospective pilot study. NAFLD diagnosis was based on ultrasonographic (US), complete clinical, anthropometric and laboratory evaluations or was confirmed by liver biopsy (two patients). The time interval between NAFLD diagnosis and participants' enrolment in the study was at least one year. During this year the participants were encouraged for changes in their lifestyle habits (dietary or physical activity habits), but without any laboratory (liver enzymes) improvement. The subjects-patients were interviewed before participating in the study using a structured form, which included sociodemographic data, lifestyle habits, and medical history. They all met the following inclusion criteria: 1) age >18 years old; 2) long history of elevated serum ALT levels (>40 IU/L), and AST/ALT <1; 3) fatty liver diagnosed by US or confirmed by liver biopsy; 4) alcohol intake <20 g per day (confirmed by at least one family member); 5) absence of other causes of serum aminotransferases chronic elevation, such as viral (B and C), autoimmune or drug-induced (e.g. lipid-lowering drugs) hepatitis, hypothyroidism, celiac disease, hereditary hemochromatosis, Wilson's disease,  $\alpha$ 1 antitrypsin deficiency, primary biliary cirrhosis and primary sclerosing cholangitis; 6)

absence of phenylketonuria (an autosomal recessive metabolic genetic disorder characterized by a mutation in the gene for the hepatic enzyme phenylalanine hydroxylase, rendering it non-functional); 7) no consumption of vitamin supplements or drugs (such as statins, omega-3 polyunsaturated fatty acids, pioglitazone, amiodarone, methotrexate, tamoxifen/synthetic estrogens, glucocorticoids, calcium channel blockers, pre and probiotics, etc.) during the last semester. After being given clear detailed explanations of the protocol and study aims, the patients gave written consent and were free to withdraw from the study at any time with no obligations. The study was conducted in accordance with the Declaration of Helsinki and was given approval by the Ethics committee of the medical research institute.

### Study design

This study was open-label, non-randomized with an intervention period of 6 months deemed sufficient to record the changes, chiefly in aminotransferases and lipid levels, before and after the supplementation of *Spirulina (A. platensis)* specifically the high quality Greek natural product (Hellenic *Spirulina Net: Production unit: Thermopigi, Sidorokastro, Serres, Greece*). Particularly, the basic nutritional components of this product were 63 g protein, 3.8 g total fat (1 g saturated fats, 2.4 g polyunsaturated fats, 0.22 g monounsaturated fats), 8.4 g carbohydrates (<0.5 g sugars) and 6.9 g dietary fiber per 100 g. Throughout the study period the participants were requested to refrain from consuming any other supplements or medication without first consulting the investigators. Blood samples were taken from the subjects after a 12-h fast at both the outset and end of the 6-month study period. Anthropometric parameters and quality of life regarding health issues were measured at each visit. *Spirulina* was orally consumed, 6 g/day (2 sachets of 3 g b.i.d.) for six months (start with 3 g/day for the first 10 days).

### Laboratory studies

Anthropometric parameters of all NAFLD cases consisting of height, body weight (BW), body mass index (BMI) and waist circumference (WC), such as arterial blood pressure (BP) measurements were recorded. WC was measured in the upright position at the level of the umbilicus. Records were made of sitting systolic and diastolic blood pressure (two measurements averaged) with a sphygmomanometer by the auscultatory method, standing body height (measured without shoes to the nearest 0.5 cm) with a rigid height meter and body weight (without shoes and tunic) with a calibrated balance scale. BMI was calculated as the weight (kg) divided by the height (m) squared ( $\text{kg}/\text{m}^2$ ). Overweight and obesity were defined as  $\text{BMI} \geq 25 \text{ kg}/\text{m}^2$  and  $\geq 30 \text{ kg}/\text{m}^2$ , respectively [14]. Resting BP  $\geq 140/90 \text{ mmHg}$  indicated hypertension. Central obesity was defined as WC >94 cm for men and >80 cm for women [15].

A complete blood count and biochemical assessments that included serum ALT, AST,  $\gamma$ -glutamyltransferase ( $\gamma$ -GT),

total cholesterol (T-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG) and fasting plasma insulin (FPI) levels, were measured at the baseline and at the end of the six-month study period. All tests were identified by standard laboratory procedures. The cut-off values of serum aminotransferases (AST, ALT) and FPI were set at 0-40 IU/L and 2.6-24.5  $\mu$ IU/mL, respectively. LDL-C and atherogenic index (AI; T-C/HDL-C ratio) were calculated using the Friedewald [16] and Lauer [17] equations, respectively. Diabetes mellitus diagnosis was according to American Diabetes Association (ADA) criteria: FPG $\geq$ 126 mg/dL, 2-h plasma glucose $\geq$ 200 mg/dL on oral glucose tolerance test (OGTT) performed with 75 g of glucose in patients with 100 mg/dL <FPG <126 mg/dL [18]. Also, a 2-h plasma glucose value 140 mg/dL to 199 mg/dL or <140 mg/dL on OGTT, was defined as impaired glucose tolerance or impaired fasting glucose, respectively [18]. The presence of IR was defined when homeostasis model assessment of IR (HOMA-IR) value was  $\geq$  2.70 [18]. HOMA-IR index was evaluated as FPI (in mIU/mL) multiplied by FPG (in mg/dL), divided by 405 [19]. The diagnosis of the MetS was based on the presence of three or more of the following criteria: 1) FPG $\geq$ 100 mg/dL; 2) central obesity [WC >94 cm (men) and >80 cm (women)]; 3) BP $\geq$ 130/85 mmHg; 4) TG levels $\geq$ 150 mg/dL; or 5) HDL-C <40 mg/dL (men) and <50 mg/dL (women) [15,20].

Measurements of health related quality of life were obtained with the Chronic Liver Disease Questionnaire (CLDQ) developed by Younossi *et al* [21,22]. The CLDQ comprises 29 items in the following six fields: abdominal symptoms, fatigue, systemic symptoms, activity, emotional function and worry. The answers to the CLDQ lead to scales of 1 to 7 which range from "1 = all of the time (worst)" to "7 = never (best)" [21,22]. The variation in the final CLDQ total score was compared to the initial score. CLDQ total score is calculated by dividing the total score by the number of items (n=29) resulting in a 1 to 7 scale [20,21]. The CLDQ is short, easy to administer and correlates with the severity of liver disease [21,22].

Among imaging techniques an abdominal US performed by an experienced abdominal radiologist was used to detect hepatic steatosis. Considering the increasing echogenicity of the liver parenchyma in comparison to that of the right kidney and diminished visualization of the diaphragm and intrahepatic vessel borders, steatosis in each patient was classed: none (normal US liver structure); mild (slight increase of echogenicity, normal visualization); moderate (diffuse increase of echogenicity, slight impaired visualization); or severe (marked increase of echogenicity, poor or no visualization) [23-25].

### Statistical analysis

Results are expressed as mean  $\pm$  standard deviation (SD). Statistical analyses were performed using GraphPad Prism 3.0 (GraphPad Software, Inc., California, USA). Comparisons between pre- and post- data were done using Student's *t* test. Paired *t* tests were used to analyze mean differences between primary and post six-month values for all measured parameters and CLDQ scores. Spearman's correlation coefficient was used

to examine the relationships between percent weight change and changes in liver chemistry, IR, and lipid profile variables. All P-values were two-tailed, and values <0.05 were considered statistically significant.

### Results

The baseline characteristics of the 15 NAFLD patients (median age: 48, range: 29-62 years) who participated in the intervention study are presented in Table 1. All patients had

**Table 1** Baseline characteristics of NAFLD patients (n=15) enrolled in the intervention study

Gender (male/female)	13/2
Age (years)	47.9 $\pm$ 9.7
Smoking	
Yes	2 (13%)
No	13 (87%)
Anthropometric values	
BW (kg)	102.9 $\pm$ 18.9
BMI (kg/m <sup>2</sup> )	32.7 $\pm$ 3.8
<18.5	0
18.5-24.9	01
25.0-29.9	02
$\geq$ 30.0	12
WC	106.5 $\pm$ 12.5
Central obesity	15 (100%)
Blood pressure	
SBP (mmHg)	127 $\pm$ 5.6
DBP (mmHg)	76.9 $\pm$ 5.2
Diabetes mellitus type 2/ IGT / IFG	3 / 3 / 4
Metabolic syndrome	15 (100%)
Fasting plasma values	
Hb (g/dL)	14.6 $\pm$ 1.1
Glucose (mg/dL)	106.7 $\pm$ 15.7
T-C (mg/dL)	275.5 $\pm$ 29.9
TG (mg/dL)	184.8 $\pm$ 16.8
HDL-C (mg/dL)	38 $\pm$ 6.9
LDL-C (mg/dL)	200.6 $\pm$ 27.6
AI	7.4 $\pm$ 1.3
AST (IU/L)	65.9 $\pm$ 23.1
ALT (IU/L)	104.4 $\pm$ 35.5
$\gamma$ -GT (IU/L)	38.2 $\pm$ 22.8
Insulin ( $\mu$ IU/mL)	17.4 $\pm$ 7.3
HOMA-IR	4.6 $\pm$ 2.1
Sonographic findings	
No steatosis	0
Mild steatosis	7
Moderate steatosis	5
Severe steatosis	3

Values are expressed as mean $\pm$ standard deviation (SD) and percentage % AI, atherogenic index; BMI, body mass index; BW, body weight; DBP, diastolic blood pressure; Hb, hemoglobin; HDL-C, high density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low density lipoprotein-cholesterol; SBP, systolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-C, total cholesterol; TG, triglycerides;  $\gamma$ -GT,  $\gamma$ -glutamyltransferase; WC, waist circumference To convert mg/dL to mmol/L: for TC, LDL and HDL multiply by 0.026; for TG by 0.0113 and for glucose by 0.0555

MetS, central obesity and normal BP. The majority of the participants (80%) were obese, but only 20% of them were diabetic (Table 1).

After the 6-month intervention period of Spirulina supplementation significant differences were observed in liver enzymes' levels. Particularly, Spirulina supplementation resulted in a significant reduction in AST levels from  $65.9 \pm 23.1$  to  $40.5 \pm 17.3$  IU/L (or -38.5%);  $P < 0.0001$ , ALT levels from  $104.4 \pm 35.5$  to  $65.3 \pm 32.3$  IU/L (or -37.5%);  $P = 0.0004$ ,  $\gamma$ -GT levels from  $38.2 \pm 22.8$  to  $28 \pm 17$  IU/L (or -26.7%);  $P = 0.0065$  (Table 2). However, no changes in sonographic findings were observed among 15 NAFLD patients after Spirulina supplementation.

Spirulina supplementation resulted in a significant reduction in T-C, TG, and LDL-C levels (-9.1%, -24.8%, -9.6%, respectively), and also in BW, AI and HOMA-IR (-8.1%, -13.5% and -19.6%, respectively). HDL-C and Hb levels were significantly increased by 4.2% and 4.1%, respectively (Table 2). No changes were observed in BP in our subjects-patients (data not presented). Percent weight change from baseline was not correlated significantly with percent changes in liver chemistry (AST, ALT,  $\gamma$ -GT), HOMA-IR, and lipid profile (T-C, TG, LDL-C, HDL-C) variables. The health-related quality of life scale, as measured by the CLDQ overall scores, was statistically significantly improved in all NAFLD patients at the end of 6 months (Table 2). No side effects, discomfort, or any other complaints were reported by any of the subjects-patients.

## Discussion

To our knowledge, this pilot study is the first human trial to address the effects of Spirulina supplementation on liver function and health-related quality of life in patients with

NAFLD. Our study yielded outcomes that showed beneficial effects of Spirulina supplementation within observed parameters on 15 NAFLD Cretan outpatients, including a significant reduction in serum liver enzymes and lipids, and additionally an improvement in scores on health-related quality of life.

In our study, Spirulina supplementation (6 g/day for 6 months) decreased AST and ALT levels by 38.5% and 37.5%, respectively. This indicates a hepatoprotective effect of Spirulina (aminotransferases reduction) on NAFLD patients, previously also recorded in three Mexican patients (one 43-year-old male with BMI 26 kg/m<sup>2</sup>; one 77-year-old male with BMI 29.9 kg/m<sup>2</sup>; and one 44-year-old female with BMI 30 kg/m<sup>2</sup>) with NAFLD (as evidenced by US and serum ALT data) and dyslipidemia [26]. The consumption of 4.5 g/day of *A. maxima* in tablet form for three months by these patients decreased the pathological levels of serum ALT (an average decrease of 41%), T-C, triacylglycerols (TAG), LDL-C and AI [26]. Several previous animal studies, but a limited number of clinical trials on humans with IR and NAFLD, have suggested that hepatoprotective properties of Spirulina are related with its antioxidant and anti-inflammatory properties (mainly due to C-phycoerythrin,  $\beta$ -carotene, and vitamin E) and the reduction in liver lipid profile [26-38]. Using a single intraperitoneal dose of 1 mL/kg carbon tetrachloride (CCl<sub>4</sub>) as a hepatotoxic in order to induce NASH, it was demonstrated that 5% *A. maxima* in the diet of rats decreases serum AST, liver TAG and T-C [31]. An identical pattern was discerned in the liver free fatty acids (with a significant decrease in unsaturated fatty acids) and the thiobarbituric acid reactive substances, indicators for lipoperoxidation [33]. Furthermore, it has been shown that *A. maxima* inhibits the development of fatty liver induced by simvastatin, ethanol and hypercholesterolemic in mice [34], and *A. fusiformis* is protective against chemical-mediated genotoxicity in mice by intensifying the activity of cellular antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase [38]. Spirulina has revealed

**Table 2** Impact of Spirulina supplementation on the studied variables (15 non-alcoholic fatty liver disease patients)

Variables	T-C (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	AI	BW (Kg)
Total (n=15)	275.5±29.9	184.8±16.8	38±6.9	200.6±27.6	7.4±1.3	102.9±18.9
Initial	250.3±26.7	138.9±23.8	39.6±6.3	183±23.6	6.4±1	94.6±17.1
Final	-9.1	-24.8	+4.2	-9.6	-13.5	-8.1
Change (%)	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
P value						

  

Variables	HOMA-IR	AST (IU/L)	ALT (IU/L)	$\gamma$ -GT (IU/L)	Hb (g/dL)	CLDQ overall score
Total (n=15)	4.6±2.1	65.9±23.1	104.4±35.5	38.2±22.8	14.6±1.1	5±0.1
Initial	3.7±1.6	40.5±17.3	65.3±32.3	28±17	15.2±0.9	6.5±0.2
Final	-19.6	-38.5	-37.5	-26.7	+4.1	+30
Change (%)	0.0003	<0.0001	<0.0001	0.0065	<0.0001	<0.0001
P value						

Data are expressed as mean±SD

AI, atherogenic index; BW, body weight; CLDQ, chronic liver disease questionnaire (29 items); Hb, hemoglobin; HDL-C, high density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low density lipoprotein-cholesterol; P, significance value, Student's-t test; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-C, total cholesterol; TG, triglycerides;  $\gamma$ -GT, gamma glutamyl transpeptidase

To convert mg/dL to mmol/L: for TC, LDL and HDL multiply by 0.026 and for TG by 0.0113



its antioxidant and/or anti-inflammatory activity in humans by reducing inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)- $\alpha$  and increasing anti-inflammatory protein adiponectin indicating a reduction of oxidative stress [28-30]. It is known that in NAFLD, inflammatory cytokines IL-6 and TNF- $\alpha$  are elevated, while adiponectin is lowered [39]. In addition, Spirulina supplementation, as a low-calorie, low-fat, and cholesterol-free source of protein, could contribute to the reduction in intrahepatic triglyceride content [40,41].

Dyslipidemia is often associated with NAFLD and regarded as a risk factor for fatty infiltration of the liver [42-44]. Our study revealed that all the undesirable lipid fractions (T-C, TG, LDL-C) were substantially reduced at the end of the 6-month period of Spirulina supplementation, with the major reduction being observed in TG levels (-24.8%). These findings are expected, considering the results of our previous study conducted in a Cretan population with the same product but at a lower dosage (1 g/day) [1]. However, the increase of HDL-C levels (4.2%) among the 15 NAFLD patients was statistically significant ( $P=0.0002$ ). The active ingredients in Spirulina that produce its hypolipidemic activity still remain unidentified. Nonetheless, it was discovered in studies on rats that an element contained in Spirulina (*A. platensis*) inhibited jejunal cholesterol absorption and ileal bile acid reabsorption, and it was suggested C-phycoerythrin produced these effects [45]. Another study using rats reported findings that a glycolipid, determined to be glycolipid H-b2 isolated from Spirulina, inhibited pancreatic lipase activity in a dose-dependent manner and also lowered postprandial TG levels [46]. The same study revealed that phycoerythrin also inhibits pancreatic lipase [46]. An alternative suggestion is that the hypolipidemic effect of Spirulina in our study could be attributed to its essential polyunsaturated (omega-6 and omega-3) fatty acids [mainly  $\gamma$ -linolenic acid (1960 mg/100 g), linolenic acid (139 mg/100 g) and  $\alpha$ -linolenic acid (311 mg/100 g)] and niacin (vitamin B3, 10.8 mg/100 g) [1]. Moreover, the significant improvement of HOMA-IR index (-19.6%) in our study patients might contribute to the reduction of LDL-C levels, considering that the expression of hepatic LDL receptors is inversely related to IR and the availability of cholesterol [44,47]. Also, it is known that BW loss alters the metabolic fate of HDL particles by decreasing plasma TG levels and delays the catabolism of HDL apoA-I with a concomitant reduction in the secretion of HDL apoA-I [44,47].

Even though current scientific evidence regarding Spirulina supplementation for BW is unsatisfactory [1], our findings revealed substantial BW loss (-8.1%) accompanied by a significant reduction of HOMA-IR index (-19.6%) following Spirulina supplementation among the 15 participants. The discrepancy between our BW findings and previous studies [1] might be due to the higher dose and longer period of administration (6 g/day for six months instead of 1g/day for 3 months). However, this BW loss is remarkable considering that a moderate BW loss (5-10% of baseline BW) has a beneficial effect on cardiovascular risk factors, such as diabetes mellitus type 2, hypertension and hyperlipidemia [48,49]. Moreover, a gradual BW loss through improved diet and increased physical activity is the main guideline for the management of NAFLD [50], the cornerstone therapy

of NAFLD, improving liver enzymes, insulin sensitivity, reducing inflammation and liver histology [6,51-56]. The BW loss could be explained mainly by the low fat and low carbohydrates content of Spirulina supplementation which is far lower than almost all other protein sources [1]. Research has demonstrated that BW loss with a low-fat diet reduces IR and cholesterol synthesis, and modifies fat liver in overweight non-diabetic subjects [40,57]. Spirulina supplementation also contains phenylalanine, a potent releaser of cholecystokinin that acts on the brain's appetite center, which in turn acts as a BW suppressant [58-60]. Although the patients were encouraged for changes in their lifestyle habits (dietary or physical activity habits) before their enrolment in our study, assessment of these habits throughout the study was not performed. So, potential changes in participants' lifestyle habits – that may have contributed to the observed weight loss – cannot be excluded. The beneficial effect of Spirulina supplementation on the haemoglobin levels in our study is due to its hematopoietic nutrients (such as protein, iron, folic acid, vitamin B12) and is known from previous studies [61-64]. The beneficial role of Spirulina and a confirmation of its safety profile were revealed with the improvement to health-related quality of life scale, the lack of adverse reactions and discomfort, and absence of complaints throughout the study.

It is known that diet is a major contributor to NAFLD. Not only the amount of energy but also the quality of the diet plays an important role in the development and progression of NAFLD [6]. Diets rich in saturated fat, cholesterol, and low in saturated fat, fiber and antioxidant vitamins C and E have been associated with NAFLD, IR and hepatic inflammation [6]. So, the composition of Spirulina (low in saturated fat and carbohydrates, rich in polyunsaturated fatty acids, vitamins and dietary fiber) makes it attractive for the management of NAFLD.

Despite the hepatoprotective effects of Spirulina supplementation on our study population, a US change of steatosis among NAFLD patients was not revealed. This finding was not surprising considering that although a US is a widely available and low-cost technique, it is not quantitative or strongly correlated with histopathologic steatosis assessment; nor is it able to display variations across steatosis grades [65,66]. Only a liver biopsy before and after Spirulina supplementation could demonstrate a change on the histopathologic grading of hepatic steatosis. Among imaging techniques, T1-weighted dual-echo MR imaging and  $^1\text{H}$  MR spectroscopy appear to have the best diagnostic accuracy in depicting hepatic steatosis, in contrast to US and CT [65]. Unfortunately, they were not available in our hospital.

Despite the limitations of the present study, i.e. pilot study, small sample size, lack of control group and assessment of dietary or physical activity habits throughout the study, no blind protocol, no histological evidence, it uncovered remarkable multiple metabolic effects which are beneficial to preventing cardiovascular disease (the most common cause of death) in outpatients with MetS and NAFLD. Thus, the role of Spirulina supplementation as a natural food supplement in NAFLD patients should not be overlooked.

## Summary Box

### What is already known:

- Spirulina is a microscopic and filamentous cyanobacterium with high nutritional value taken as a safe dietary supplement for both humans and animals
- Species of Spirulina have exhibited metabolic (hypolipidemic, hypoglycemic), anti-viral, liver-protecting and blood-vessel relaxing effects, anti-cancer, anti-inflammatory and anti-oxidant properties
- Proven effective therapies for the management of non-alcoholic fatty liver disease (NAFLD) do not exist. The cornerstone therapy of NAFLD is directed toward weight loss, lifestyle modifications, and comorbidity management

### What the new findings are:

- Spirulina (*Arthrospira platensis*) supplementation at a high dosage of 6 g daily in NAFLD patients has strong and multiple beneficial metabolic effects and improves their health-related quality of life
- Spirulina supplementation could be used as a safe and effective dietary supplement on NAFLD patients

In conclusion, the results of this study demonstrate that Spirulina (*A. platensis*) supplementation at a high dose of 6 g daily exhibits marked and multiple beneficial metabolic effects and boosts health-related quality of life. We suggest that Spirulina supplementation could be used as a dietary supplement on NAFLD patients. Further studies will be necessary to determine the mechanism(s) of Spirulina's action on metabolic parameters.

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