

Effects of High Fat Diets and *Spirulina platensis* Supplementation in New Zealand White Rabbits

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Abstract: The present study was designed to examine, whether rabbits fed a diet containing High Fat (HF) could develop obesity and be predisposed to developing metabolic syndrome (Mets). The results have shown an increased adipose accumulation with a significant weight gain and an increase in abdominal circumference, periscapular fat and liver weight in rabbits fed HF diets compared to rabbits fed a Low Fat (LFC) diet, associated with higher levels of plasma glucose, insulin and lower levels of High-Density-Lipoprotein (HDL) cholesterol in the rabbits fed the HF diets than the rabbits fed the LFC diet. An Oral Glucose Tolerance Test (OGTT) has been performed to evaluate glucose metabolism; the plasma glucose levels in the rabbits fed the HF diets were constantly higher (statistically significant at 0, 60, 90, 120 and 240 min) than in the rabbits fed the LFC diet. The association between the HF diets and oxidative stress, indicated by the presence of Reactive Oxygen Species (ROS) in plasma, has also been investigated; the rabbits fed the HF diets had higher ROS values than the rabbits fed the LFC diet. In addition, the protective effect of *Spirulina platensis* (SP), antioxidant of vegetable origin, has also been investigated. The SP supplementation (10 g kg⁻¹ of the diet) did not have any effect on the morphological data or some parameters in plasma, while SP was able to reduce the ROS value in rabbits fed the high fat diet probably due to beneficial effect of the γ -linolenic acid content in the SP.

Key words: Obesity, *Spirulina platensis*, metabolic syndrome, rabbits, fats, plasma

INTRODUCTION

In western countries, obesity has reached epidemic dimensions and its incidence is on the rise (Meerarani *et al.*, 2006). The excessive amount of nutritive substances (particularly fats) together to the excess of fat reserve in the organic tissues, is one of the main causes of obesity. Overweight is a predisposing cause of cancer, type 2 diabetes and cardiovascular disease; moreover, the average life expectancy of obese individuals is reduced by 9 years (Caballero, 2003). The scientific community is currently paying more and more attention to the serious health problem of overweight in the general population and its related complications. Several studies have suggested a link between high fat diets, abnormalities in fat metabolism and obesity (Carroll and Tyagi, 2005). Furthermore, obesity is a main causative factor in the development of the metabolic syndrome (Mets), a disorder that is defined in various ways by

Skurk and Hauner (2004). The original description was given by Reaven (1988), who characterized it as involving a weight gain, an increase in abdominal circumference, derangement of the lipids metabolism (linked to a low level of high-density-lipoprotein cholesterol) and derangement of the glycidic metabolism (linked to a high levels of plasma glucose and insulin), which can determine the insulin resistance. Recent US statistics confirm that Mets affects roughly 25% of young people and up to 45% of the population over 50 years and that the incidence of Mets is sharply increasing with the growing population of overweight individuals with visceral adiposity due to overeating and to a high fat diet (Muredach *et al.*, 2003; Flier, 2004).

The correlation between obesity, Mets and oxidative stress has been known for several years. Obesity, which is commonly due to excessive consumption of high fat foods, is associated with a deficiency of antioxidant defence capacity and an increase in oxidative stress. In

the presence of obesity and Mets, the overproduction of the superoxide anion by the activation of NADPH oxidase, produces Reactive Oxygen Species (ROS) (Delbosc *et al.*, 2005; Wautier *et al.*, 2001). Superoxides can activate several damaging pathways that can be a key-event in the initiation and development of vascular complications. In spite of the excess caloric intake, antioxidant vitamin levels decrease in both obese children and adults (Kljno *et al.*, 1998). The reduced antioxidant vitamin levels mechanism observed in association with obesity and Mets still remains unclear.

The finding of decreased serum antioxidant levels in obese individuals raises the question of whether, antioxidant supplements should be recommended in the setting of a high fat diet. In light of its clinical significance, the development of therapeutic remedies for obesity and Mets is of primary importance. The management of obesity and Mets basically focuses on therapeutic lifestyle modifications, including weight reduction and improved diet. Furthermore, the positive role of antioxidants in the reduction of the damage caused by free radicals during the pathology is unquestionable. Some antioxidants of vegetable origin can help a management of obesity and the connected complications such as diabetes and Mets, due to their protective properties, which help counteract free radical damage and to their hypolipidemic and hypocholesterolemic properties (Honjo and Inoue, 2006).

Systematic screening for therapeutic substances derived from algae, particularly cyanobacteria (blue-green algae), has received great attention recently as a nutritional supplementation strategy geared towards the prevention of the health problems such as obesity, Mets and the complications that are connected with free radical damage.

The blue-green algae *Spirulina platensis* (SP), with high concentrations of functional nutrients, is a natural antioxidant and the subject of extensive research in this field (Henrikson, 1997). SP contains essential aminoacids, phenolic acids, essential fatty acids, vitamins (tocopherols, beta-carotene), pigment (C-Phycocyanin) and enzymes (superoxide dismutase), which are known to exhibit antioxidant properties (Dasgupta *et al.*, 2001; Manoj and Venkataraman, 1992; Lissi *et al.*, 2000; Bhat and Madyastha, 2000). SP may be used as a therapeutic supplement for the management of various nutritional and metabolic disorders (Mani *et al.*, 2000); SP extracts in fact produce antiobesity, lipid lowering, hypocholesterolemic and antioxidant effects (Richmond, 1992; Iwata *et al.*, 1990; Nakaya *et al.*, 1988; Miranda *et al.*, 1998).

SP is one of the few sources of dietary γ -linolenic acid (GLA, C18:3n-6), which can be considered as a signalling molecule, with agonist effects on transcriptional factors, which are currently implicated as key players in adipocyte differentiation and functions, including Peroxisomal Proliferator-Activated Receptors (PPARs) (Madsen *et al.*, 2005).

In vivo studies on both preventive and therapeutic strategies of *Spirulina platensis* towards the alterations of the lipidic metabolism, have been based themselves on animal models. The metabolic characteristics inherent to rabbits suggest the feasibility of an animal model of human disease with complex manifestations, such as obesity and Mets Kawai *et al.* (2006). As far lipid metabolism is concerned, rabbits have several advantages over other species; they in fact have higher levels of apoB-containing low density lipoproteins than mice or rats. Furthermore, the lipoprotein profile of rabbits is more like that of humans than mice and a pattern of hepatic apo B100 and intestinal apo B48 synthesis that resembles that of humans. Like humans, but unlike mice, rabbits have an abundant cholesteryl ester transfer protein, an important regulator of reverse cholesterol transport (Brousseau and Hoeg, 1999; Ichikawa *et al.*, 2004).

The aim of the present research was to evaluate the variation induced in rabbits by a diet with a high fat content. In particular, the tendency of the subjects fed fat diets to develop an obesity condition, disorders of glucose metabolism and oxidative stress, which can mimic human Mets, has been evaluated. Furthermore, we studied the protective effects of *Spirulina platensis* supplementation in these subjects.

MATERIALS AND METHODS

Animals and diets: Male New Zealand white rabbits (Harlan-Italy, Udine, Italy), weighing on average 2805 ± 65 g were kept with the Principles of Laboratory Animal Care (NIH no. 85-23, revised 1985) randomly assigned to 4 groups with equal initial weight variability. The animals were kept in individual cages in an animal room (temperature $22 \pm 2^\circ\text{C}$) in a 12 h dark-light cycle. The experimental period lasted 3 month with an adaptation period of 2 weeks. The rabbits in each group were fed 100 g of the following diets/day.

- Low Fat Control (LFC) group (n = 4): standard rabbit diet
- Low Fat Spirulina (LFS) group (n = 4): standard rabbit diet supplemented with *Spirulina platensis* (1%)

- High Fat (HF) group (n = 4): high fat diet (consisting of the standard rabbit diet with 10% added fat: 6.7% corn oil and 3.3% lard)
- High Fat Spirulina (HFS) group (n = 4): high fat diet supplemented with *Spirulina platensis* (1%)

All the experimental diets were prepared by Laboratorio Dottori Piccioni (Gessate, Italy) and formulated to meet all the essential nutrient requirements for rabbits according to the National Research Council (1977). They also contained the recommended levels of vitamins and minerals for rabbits. The standard rabbit diet contained the following ingredients: corn, barley, wheat grain, dehydrated alfalfa meal, soybean seed meal, wheat red shorts, carob pulp, torula yeast, dicalcium phosphate, calcium carbonate, salt and magnesium oxide. These diets were identical in composition except for the SP supplementation (0 and 1%) and level of fat (3 and 13%). The four diets were sampled during the trials to determine the chemical composition, gross energy and Fatty Acid (FA) profile.

The rabbits were weighed individually at the beginning and at the end of the experiment. Mortality was recorded daily. One week before the end of the trial, after over-night fasting, an oral glucose tolerance test was carried out. Body weigh and abdominal circumference of the rabbits were measured at the end of the experiment and all the rabbits were then killed, after anesthetization with Zoletil 100 (Tiletamine-Zolarepam, Virbac, Carros; France), by aortic exsanguination. The blood was collected and plasma and serum were isolated. During the necropsy the liver and periscapular fat were removed and weighed. Moreover, *longissimus dorsi* muscle and perirenal fat samples were collected to determine their FA profile.

Proximate analysis and gross energy determination of the diets: The diet samples were analyzed to determine dry matter, Total Nitrogen (TN) content (AOAC, 1990), ash by ignition to 550°C, Ether Extract (EE) using the Soxhlet method (AOAC, 1990), neutral detergent fibre without sodium sulfite and α -amylase and acid detergent fibre as described by Van Soest *et al.* (1991) expressed exclusive of residual ash, lignin determined by solubilization of cellulose with sulphuric acid as described by Robertson and Van Soest (1981) and Gross Energy (GE), which was determined using an adiabatic bomb calorimeter (IKA C7000, Staufen, Germany).

Glucose levels measurement and oral glucose tolerance test: The glucose levels and Oral Glucose Tolerance Test (OGTT) were tested using an Accu-Check Compact kit

(Roche Diagnostics, Gmbh, Mannheim, Germany). After a fasting period of 12 h, a 50% glucose solution was orally administrated to the animals at a dose of 1.5 g kg⁻¹. Blood samples were collected through the auricular artery before the test and 15, 30, 45, 60, 90, 120 and 240 min after glucose loading. The incremental Area Under the Curve (AUC) was calculated by multiplying the cumulative mean height of glucose by the time (Zhao *et al.*, 2008).

Insulin, HDL-cholesterol and ROS measurements: Plasma insulin was measured using an ultrasensitive enzyme-linked immunosorbent assay kit (DRG Diagnostics, Marburg, Germany).

Plasma HDL-cholesterol was determined according to standard enzymatic procedures using reagent kits (Hospitex Diagnostic, Florence, Italy).

ROS in plasma were measured using 2',7'-dichlorofluorescein diacetate as a probe (Ravindranath, 1994).

Fatty acid profile of the diets, longissimus dorsi muscle and perirenal fat: The lipid extraction was performed on the diets, meat and fat samples according to Hara and Radin (1978), while the transesterification of the FAs was carried out according to Christie (1982), with the modifications described by Chouinard *et al.* (1999).

The FAs were analyzed as their methyl esters. The analysis was carried out by gas chromatography, using a Dani GC 1000 DPC (Dani Instruments S.P.A., Cologno Monzese, Italy), equipped with a flame ionisation detector and a PTV injection port used in the split mode with a split ratio 1:50. The injector and detector ports were set at 245 and 270°C, respectively. The oven temperature program was initially set at 50°C for the first min and then increased at a rate of 15°C min⁻¹ to 200°C, where it remained for 20 min and then increased at a rate of 5°C min⁻¹ to 230°C, where it remained for the last 3 min. The carrier gas was hydrogen. The FA methyl esters were separated with a fused silica capillary column-Supelcowax-10 (60 m×0.32 mm (i.d.), 0.25 µm). One microlitre was injected using a Dani ALS 1000 auto sampler. The peak area was measured using a Dani Data Station DDS 1000 and where each peak was identified and quantified by pure methyl ester standards (Restek Corporation, Bellefonte, PA, USA).

The Saturation (S/P) indexes were calculated according to Ulbricht and Southgate (1991) as follows:

$$S/P = (C14:0 + C16:0 + C18:0)/MUFA + \Sigma PUFA$$

where, MUFA and PUFA are monounsaturated FAs and polyunsaturated fatty acids, respectively.

Statistical analysis: The statistical analyses were performed using the STATA software package (version 9.2 for Windows, STATA Corp, USA). Non parametric analysis of variance (Kruskal-Wallis test) was used to evaluate the effects of diets on the morphological data and some parameters in plasma. Each pair of groups of rabbits (HF vs. LFC, HFS vs. LFC and HF vs. HFS) was also compared using the Mann-Whitney test.

The differences in the FA profiles of the meat and perirenal fat samples of the rabbits were then tested separately for the rabbits fed the LF diets (LFC vs. LFS) and rabbits fed the HF diets (HF vs. HFS) of animals using the Mann-Whitney test.

RESULTS

General features: The chemical composition and gross energy of the diets and the vitamin and mineral supplementation are reported in Table 1. The feedstuffs were formulated to obtain similar caloric values for the two LF diets and the two HF diets.

The effects of the diets on the morphological data and some parameters in plasma of the four rabbit groups are shown in Table 2.

All the rabbits had equivalent initial live weight values. The final body weight and abdominal circumference of the rabbits fed the HF and HFS diets were significantly ($p < 0.05$) increased compared to the rabbits fed the LFC diet after three months of treatment. These data suggested that central obesity with perivisceral fat accumulation existed in the HF and HFS rabbits. Rabbits fed the HFS diet showed a similar body weight and abdominal circumference to the rabbits fed the HF diet.

The periscapular fat weight of the rabbits fed the HF and HFS diets was significantly ($p < 0.05$) increased compared to the rabbits fed the LFC diet. Rabbits fed the HFS diet showed a similar periscapular fat weight to the rabbits fed the HF diet.

The liver weight of the rabbits fed the HF diet was significantly ($p < 0.05$) greater than that of the rabbits fed

the LFC diet. However, the values of the liver weight of the rabbits fed the HFS diet not show any significant differences from those of the rabbits fed the LFC and HF diets.

As far the parameters in plasma are concerned (Table 2), the insulin values in the HF and HFS groups were significantly ($p < 0.05$) higher than the LFC group, while the rabbits fed the HFS diet showed similar insulin values to the rabbits fed the HF diet.

The HDL-cholesterol values belonging to the HF and HFS groups of rabbits were significantly ($p < 0.05$) lower than the LFC group, while the rabbits fed the HFS diet showed similar HDL-cholesterol values to the rabbits fed the HF diet.

The glycemia values in both the HF and HFS groups were significantly ($p < 0.05$) higher than in the LFC group, while the rabbits fed the HFS diet showed similar glucose values to the rabbits fed the HF diet. These findings are confirmed by the developments of the OGTT illustrated in Fig. 1. The plasma glucose levels in the HF and HFS rabbits were constantly higher and statistically ($p < 0.05$) significant at baseline, 60, 90, 120, 240 min, than the LFC rabbits. The rabbits fed the HFS diet showed a similar OGTT values to the rabbits fed the HF diet.

The AUC of the glucose in the HF and HFS rabbits was significantly ($p < 0.01$) larger than in the control rabbits (Fig. 1). However, the values of AUC of

Table 1: Chemical composition (g/100g DM) and gross energy (MJ kg⁻¹ DM) of the Low Fat Control (LFC), Low Fat + Spirulina (LFS), High Fat (HF) and High Fat + Spirulina (HFS) diets

Parameters	LFC	LFS	HF	HFS
Dry matter	92.4	92.6	94.2	94.7
Organic matter	90.5	90.6	91.4	91.3
Crude protein	19.1	18.4	16.3	17.0
Ether extract	3.1	3.1	12.5	13.5
Crude ash	9.5	9.4	8.6	8.7
Neutral detergent fibre	34.3	34.0	33.0	32.5
Acid detergent fibre	18.5	18.4	17.4	15.9
Acid detergent lignin	3.2	3.1	3.1	3.5
Gross energy	18.1	18.5	19.9	20.1

Vitamins and minerals/kg diet: Vit. A 18000 UI; Vit. D₃ 1800 UI; Vit. E 40 mg; Vit. B₁ 8 mg; Vit. B₂ 10 mg; Vit. B₆ 8 mg; D-pantothenic acid 10 mg; Vit. K 2 mg; Niacin 20 mg; Vit. B₁₂ 0.2 mg; Folic acid 2 mg; Choline 1 g; Biotin 0.1 mg; DL-methionine 500 mg; J 0.6 mg; Mn 45 mg; Co 0.25 mg; Fe 50 mg; Zn 40 mg; Cu 5 mg

Table 2: Effects of diet on the morphological data and parameters in plasma of rabbits fed the Low Fat Control (LFC), Low Fat + Spirulina (LFS), High Fat (HF) and High Fat + Spirulina (HFS) diets^a

Parameters	Kruskal wallis significance	LFC	LFS	HF	HFS
Initial weight (g)		2791±44	2794±107	2836±27	2799±72
Final Weight (FW; g)	¥	3155±116	3134±110	3535±150*	3374±150*
Abdominal circumference (cm)	¥	33.0±0.8	33.3±1.5	37.5±1.3*	36.8±1.8*
Periscapular fat weight (g)	¥	11.0±5.8	10.7±4.7	30.3±2.7*	26.9±9.9*
Liver weight (g)		71.4±5.7	72.6±6.7	80.5±6.3*	73.3±7.5
Insulin (ng mL ⁻¹)	¥	2.4±0.3	4.1±3.2	15.2±2.3*	13.9±5.2*
HDL-cholesterol (mg mL ⁻¹)	¥¥	24.2±2.7	24.2±4.0	18.2±1.2*	19.0±0.6*
Glucose (mmol L ⁻¹)	¥¥	6.2±0.2	6.6±0.3	9.1±0.2*	9.2±0.1*

^aThe data are means (n = 4 for each group)±SD. Statistical significance: * $p < 0.05$ HF vs. LFC and HFS vs. LFC, Kruskal Wallis significance: ¥ $p < 0.05$, ¥¥ $p < 0.01$

Table 3: Fatty acid (g/100g of total FA) pattern of the Low Fat Control (LFC), Low Fat + Spirulina (LFS), High Fat (HF) and High Fat + Spirulina (HFS) diets

Parameters	LFC	LFS	HF	HFS
C14:0	0.3	0.2	0.5	0.5
C16:0	15.7	15.2	15.7	15.6
C16:1	0.3	0.5	0.8	0.7
C17:0	0.7	0.4	0.2	0.2
C17:1	0.0	0.0	0.1	0.1
C18:0	2.4	2.4	5.8	5.8
C18:1	21.3	19.9	31.7	31.3
C18:2n-6	50.7	50.1	41.2	41.8
C18:3n-3	7.3	9.0	2.3	2.2
C18:3n-6	0.0	0.7	0.0	0.2
C20:0	0.0	0.3	0.3	0.3
C20:1n-9	0.6	0.5	0.5	0.4
C20:2n-9	0.0	0.0	0.3	0.2
Other	0.7	0.7	0.7	0.6
SFA ^a	19.1	18.6	22.5	22.4
MUFA ^b	22.1	20.9	33.0	32.6
PUFA ^c	58.0	59.1	43.7	44.2
n-6/n-3 ^d	6.9	5.5	17.9	19.2

^aSFA: Saturated Fatty Acid. ^bMUFA: Monounsaturated Fatty Acid. ^cPUFA: Polyunsaturated Fatty Acid. ^dn-6/n-3: PUFA n-6/ PUFA n-3 ratio

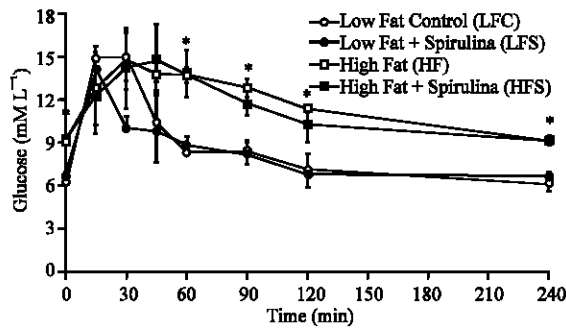


Fig. 1: Comparison of plasma glucose levels before and 15, 30, 45, 60, 90, 120 and 240 min after glucose loading in the rabbits fed the Low Fat Control (LFC, 1896.2±69.6), Low Fat + Spirulina (LFS, 1980.2±134.4), High Fat (HF, 2655.6±210.8**) and high fat + spirulina (HFS, 2770.9±33.2**) diets. The Area Under the glucose Curve (AUC) was calculated by multiplying the cumulative mean height of glucose by time (min). Statistical significance: *p<0.05 HF vs. LFC and HFS vs. LFC, **p<0.01 HF vs. LFC and HFS vs. LFC

the rabbits fed the HFS diet did not show any significant difference from those of the rabbits fed the HF diet.

Oxidative parameters: Figure 2 shows, the plasma ROS values in the 4 rabbit groups and some very interesting results. The rabbits fed the HF and HFS diets had significantly (p<0.05) higher ROS values in plasma than the rabbits fed the LFC diet; however, the rabbits fed the HFS diet showed significantly (p<0.05) lower ROS values than the rabbits fed the HF diet.

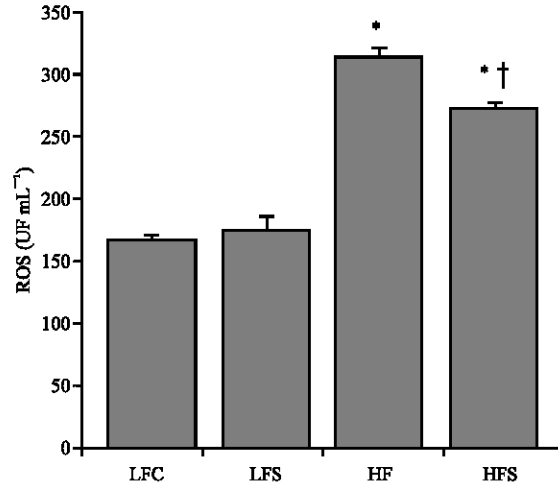


Fig. 2: Reactive Oxygen Species (ROS) values in the rabbits fed the Low Fat Control (LFC), Low Fat + Spirulina (LFS), High Fat (HF) and High Fat + Spirulina (HFS) diets. Statistical significance: *p<0.05 HF vs. LFC and HFS vs. LFC, †p<0.05 HFS vs. HF

Fatty acid profile: The FA pattern of the diets is reported in Table 3. The LFC and LFS diets showed lower SFA and MUFA contents than those of the HF and HFS diets, due to the low content of stearic acid (C18:0) and oleic acid (C18:1), respectively, while in the LFC and LFS diets, the PUFA content was higher than those of the two HF diets, due to the high content of linoleic (C18:2n-6) and α -linolenic acid (ALA, C18:3n-3).

Moreover, the n-6/n-3 ratio in the two HF diets was higher than those of two LF diets, due to their low content of ALA.

The FA composition of the *longissimus dorsi* muscle and perirenal fat of the rabbits are reported in Table 4 and 5.

Table 4 shows, significant differences (p<0.05) in the *longissimus dorsi* FAs of the rabbits fed the two LF diets (LFS vs. LFC), for the ALA content and for the n-6/n-3 PUFA ratio.

Table 5 shows, some significant differences (p<0.05) in the perirenal fat fatty acids of the rabbits fed the two LF diets, for the ALA and GLA contents and for the n-6/n-3 PUFA ratio; similar differences were also found in the perirenal fat of the rabbits fed the two HF diets (HFS vs. HF), for the GLA content.

The GLA content of the perirenal fat of the rabbits fed the LFS diet was significantly (p<0.05) greater than that of the rabbits fed the LFC diet; similar results were found for the GLA content of perirenal fat of the rabbits fed the two HF diets (HFS significantly higher than HF; p<0.05).

Table 4: Effects of diet on fatty acid composition (g/100 g of total FA) in the *Longissimus dorsi* muscle of rabbits fed the Low Fat Control (LFC), Low Fat + Spirulina (LFS), High Fat (HF) and High Fat + Spirulina (HFS) diets

Parameters	LFC	LFS	HF	HFS
C14:0	2.30±0.30	2.22±0.31	1.75±0.25	1.65±0.13
C14:1	0.33±0.10	0.25±0.19	0.27±0.06	0.26±0.12
C15:0	0.45±0.03	0.42±0.05	0.30±0.02	0.30±0.03
C16:0	26.13±0.82	26.39±1.15	21.51±0.62	21.36±1.23
C16:1	5.78±1.02	5.22±1.69	4.60±0.69	4.43±1.24
C17:0	0.56±0.02	0.52±0.07	0.37±0.03	0.39±0.07
C17:1	0.41±0.04	0.36±0.1	0.27±0.02	0.28±0.02
C18:0	6.10±0.23	6.00±0.37	5.30±0.27	5.97±0.74
C18:1	28.68±0.69	28.44±3.83	31.75±0.33	31.73±1.37
C18:2n-6	20.56±1.24	20.92±2.52	28.66±0.70	27.21±2.59
C18:3n-3	2.43±0.34	2.36±0.63*	1.97±0.18	1.85±0.29
C18:3n-6	0.00±0.00	0.09±0.11	0.00±0.00	0.08±0.15
C21:0	4.52±1.57	4.97±2.34	2.11±0.57	3.46±1.06
Other	1.75±0.64	1.84±0.76	1.13±0.37	1.03±0.31
SFA ^b	40.07±0.99	40.51±3.19	31.35±0.40	33.13±2.52
MUFA ^c	35.20±1.64	34.27±5.74	36.91±0.73	36.70±2.70
PUFA ^d	22.98±1.11	23.28±2.28	30.62±0.65	29.05±2.87
n-6/n-3 ^e	8.61±1.39	9.44±2.93*	14.66±1.40	14.87±0.90
S/P ^f	0.59±0.02	0.60±0.06	0.42±0.01	0.44±0.04

^aThe data are means (n = 4 for each group)±SD. ^bSFA: Saturated Fatty Acid; ^cMUFA: Monounsaturated Fatty Acid; ^dPUFA: Polyunsaturated Fatty Acid; ^en-6/n-3: PUFA n-6/PUFA n-3 ratio; ^fS/P: Saturated fatty acid/unsaturated fatty acid. Statistical significance for the Low Fat (LF) diets: *p<0.05 LFS vs. LFC

Table 5: Effects of diet on the fatty acid pattern (g/100 g of total FA) of the perirenal fat of rabbits fed the Low Fat Control (LFC), Low Fat + Spirulina (LFS), High Fat (HF) and High Fat + Spirulina (HFS) diets^a

Parameters	LFC	LFS	HF	HFS
C10:0	0.03±0.01	0.03±0.02	0.02±0.01	0.00±0.00
C14:0	2.03±0.12	2.05±0.23	1.33±0.23	1.13±0.09
C14:1	0.16±0.04	0.16±0.02	0.08±0.03	0.06±0.04
C15:0	0.59±0.04	0.55±0.05	0.33±0.03	0.32±0.03
C16:0	25.64±0.77	25.41±0.79	20.17±1.54	18.29±1.10
C16:1	3.08±0.47	3.19±0.13	2.01±0.46	1.74±0.44
C17:0	0.73±0.06	0.68±0.02	0.43±0.03	0.44±0.06
C17:1	0.34±0.03	0.32±0.02	0.24±0.02	0.2±0.03
C18:0	6.90±0.51	6.44±0.32	6.37±0.62	6.62±0.99
C18:1	24.83±0.46	25.48±2.45	30.74±0.83	30.47±1.21
C18:2n-6	28.76±1.83	28.27±1.94	32.9±1.420	35.29±1.39
C18:3n-3	3.37±0.23	3.92±0.22*	2.43±0.20	2.31±0.19
C18:3n-6	0.22±0.04	0.30±0.05*	0.12±0.01	0.16±0.02†
C20:0	0.19±0.05	0.15±0.02	0.15±0.02	0.16±0.05
C20:1n-9	0.45±0.08	0.41±0.10	0.44±0.06	0.35±0.05
C20:2n-6	0.25±0.03	0.24±0.04	0.29±0.04	0.25±0.04
C20:4n-6	0.06±0.02	0.07±0.02	0.05±0.00	0.02±0.03
C21:0	0.13±0.02	0.11±0.02	0.11±0.00	0.12±0.02
Other	2.25±0.18	2.23±0.12	1.77±0.07	2.07±1.03
SFA ^b	36.22±0.91	35.41±0.68	28.92±1.92	27.08±0.91
MUFA ^c	31.95±1.33	32.74±2.72	35.52±1.30	34.55±2.16
PUFA ^d	32.66±1.73	32.79±1.98	35.8±1.23	38.04±1.40
n-6/n-3 ^e	8.75±1.08	7.38±0.60*	13.81±1.64	15.56±1.31
S/P ^f	0.53±0.02	0.52±0.02	0.39±0.04	0.36±0.02

^aThe data are means (n = 4 for each group) ±SD. ^bSFA: Saturated Fatty Acid; ^cMUFA: Monounsaturated Fatty Acid; ^dPUFA: Polyunsaturated Fatty Acid; ^en-6/n-3: PUFA n-6/ PUFA n-3 ratio; ^fS/P: Saturated Fatty Acid/Unsaturated Fatty Acid. Statistical significance for the Low Fat (LF) diets: *p<0.05 LFS vs. LFC. Statistical significance for the High Fat (HF) diets: †p<0.05 HFS vs. HF

DISCUSSION

Obesity is the direct results of an imbalance between energy intake and energy expenditure. The excess energy

is primarily stored in adipose tissue in the form of triglycerides. Although, adipocytes are specifically designed to store energy, the morphological changes associated with adipose tissue growth are not without consequences for the organism as a whole (Jernas *et al.*, 2006).

The diet induced obese animal model could provide a useful tool to investigate the pathophysiological mechanism of Mets (Carroll *et al.*, 1996).

Studies in rabbits by Carroll and Tyagi (2005) have shown the development of obesity associated with hypertension in rabbits fed for 12 weeks with 10% fat added in standard diets. The research confirms these results; the rabbits fed for 3 months with the HF diet show a significant increase in morphological parameters compared to the rabbits fed the LFC diet.

Moreover, in the study, an enlarged abdominal circumference and marked visceral fat accumulation were also, noted in the rabbits fed the HF diet compared to the rabbits fed the LFC diet.

Visceral obesity, which is characterized by excess fat storage in and around the abdomen, has been classified by the World Health Organization as a physical characteristic that is specific to Mets and is the prime cause of metabolic abnormalities related to it. Accordingly, the rabbits fed the HF diet can be considered a model that closely resembles a type of central obesity and Mets in humans (Kawai *et al.*, 2006). Accumulated evidence suggested that the adipose tissue function is compromised in response to adipocyte hypertrophy during the development of obesity and this also causes structural and metabolic alterations in some organs of the body, including hepatic tissue (Stienstra *et al.*, 2007).

The study has shown that rabbits fed the HF diet have increased liver weight compared to rabbits fed the LFC diet. Many studies have confirmed these results and shown that obesity is linked to an increase in the liver weight due to the fat storage in the liver and this is currently considered a major risk for the development of Non Alcoholic Fatty Liver Disease (Stienstra *et al.*, 2007), which is one of the most common chronic and progressive liver diseases in developed countries, with the number of people affected increasing rapidly (Clark *et al.*, 2002).

However, in the study we did not find any significant effect of SP supplementation on the morphological parameters of the rabbits fed the HFS diet compared to the rabbits fed the HF diet. The results require further histopathological studies, because they have not confirmed the lipid lowering function and the regulatory effect of SP on lipid metabolism described by several other researchers, who reported that the properties of SP increases the activity of lipoprotein lipase or its role on adipocyte differentiation (Richmond, 1992; Iwata *et al.*,

1990). Several studies have in fact shown that GLA contained in the SP is able to suppress lipogenic gene transcription because of the property of PPAR activators/ligands (Madsen *et al.*, 2005). Furthermore, the study does not confirm the effects of SP on reducing the liver weight and consequently on preventing the formation of hepatic steatosis described in mice with experimental diabetes (Rodriguez-Hernandez *et al.*, 2001) or in rats after the administration of a 60% fructose based diet (Gonzalez de Rivera *et al.*, 1993).

The results obtained by these researchers support the hypothesis that SP suppresses fat liver storage by stimulating peroxisomal β -oxidation of FAs (Belay, 2002).

As far as, the parameters in plasma are concerned, we found that in addition to having central obesity, the HF rabbits exhibited impairment of glucose metabolism, because their glucose and insulin plasma levels were significantly higher than the rabbits fed the LFC diet. Moreover, the OGTT values and the AUC of glucose were significantly greater than those of the control rabbits, as was observed by Zhao *et al.* (2008).

Furthermore, the observations confirm the data reported by several researchers, who have stated that there is a positive correlation between the accumulation of visceral fat and an increase in fasting plasma glucose and insulin levels (Kobayashi *et al.*, 2001). The mechanism of these abnormalities in both glucose and insulin metabolism is not completely known, but previous studies showed that visceral adipose tissue of obese animals and humans with Mets could lead to increased free FAs influx through the portal vein into the liver, which could result in a state of insuline resistance (Meerarani *et al.*, 2006; Hotamisligil *et al.*, 1993; Hotamisligil *et al.*, 1996). Moreover, many of the inflammatory markers found in the plasma of obese individuals appears to originate from adipose tissue (Alberti and Zimmet, 1998; Maeda *et al.*, 2002).

The research shows that the HF diet not only results in an increase in plasma glucose and insulin, but also in lower HDL-cholesterol values, indicating a condition of dyslipidaemia Eckel *et al.* (2005). There are many metabolic causes that can lower HDL-cholesterol, for example, hepatic lipoprotein lipase and endothelial lipase increase the hydrolysis of HDL-cholesterol resulting of in low HDL-cholesterol levels (Broedl *et al.*, 2005). As far the effect of SP supplementation on the parameters in plasma, in the researches we did not fund any significant differences in the rabbits fed the HFS diet compared to the rabbits fed the HF diet. Therefore, the research reveals that the SP supplementation does not reduce the hyperglycemia and hyperinsulinemia in rabbits fed a HF diet and does not protect these subjects from the appearance of dyslipidaemia, as indicated by the low HDL-cholesterol values.

In a human clinical study, a significant decrease in the fasting plasma sugar level of diabetic patients was observed after 21 days of 2 g day⁻¹ SP supplementation (Mani *et al.*, 2000). Moreover, in a study involving rats, Iwata *et al.* (1987) found that feeding Spirulina at 5, 10 and 15% of the diet resulted in a significant inhibition of the total cholesterol, triglycerides and phospholipids levels in fructose-induced hyperlipidemic rats. Similar results have been found in other studies (Takai *et al.*, 1991).

It is therefore, possible that the dose of SP used in the study was too low to reduce the high value of glucose and insulin induced by the HF diet and too low to have HDL-cholesterol regulatory properties. As far the assessment of oxidative stress, the data confirm the data from previous studies about the relationship between HF diets and the increased presence of ROS in plasma (Meerarani *et al.*, 2006; Roberts *et al.*, 2006). Several theories explain the association between HF and oxidative stress. For example, the oxidative stress in rats fed HF diets may be due to upregulation of NADPH oxidase, a major source of ROS in the kidney and cardiovascular tissue (Vaziri, 2004). NADPH oxidase catalyses the transfer of a single electron to molecular oxygen to produce superoxide. The role of oxygen-derived species in causing cell injury or death is increasingly recognized: superoxide and hydroxyl radicals are involved in a large number of degenerative changes, often associated with an increase in peroxidative processes and linked to low antioxidant concentration (Halliwell and Gutteridge, 1989). Oxidative stress can in fact result from either an excess of ROS production and/or a deficient antioxidant capacity. Some researchers have found that the consumption of HF diet results in downregulation of antioxidant vitamin (β -carotene and α -tocopherol) levels and several key antioxidant enzymes: superoxide dismutases, glutathione peroxidase, catalase, heme oxidase (Roberts *et al.*, 2006).

The finding of decreased serum antioxidant levels in obese individuals raises the question of whether, antioxidants supplements should be recommended in the setting of a HF diet. The antioxidant properties of SP and its extracts have recently attracted the attention of researchers in this field (Belay, 2002). In a study by Miranda *et al.* (1998), the antioxidant activity of a blue-green algae methanolic extract was determined *in vitro* and *in vivo*. The research highlights the effect of SP in countering the increase of ROS in animal induced by HF diets, due to the SP antioxidant action. The antioxidant effect was attributed to GLA, beta carotene, tocopherol and phenolic compounds working individually or in synergy. GLA, a component of SP, has been considered to have antioxidant and anti-cancer properties (Dasgupta *et al.*, 2001). In the research, the FA profile of the perirenal fat of rabbits fed the two diets supplemented with SP had a significantly ($p < 0.05$) higher content of GLA

than the rabbits fed the two diets without SP (LFS vs. LF and HFS vs. HF). The antioxidant role of the GLA could be due to its function as a PPARs activator (Madsen *et al.*, 2005). The activation of PPARs by GLA could be a common mechanism that could explain the beneficial effects of *Spirulina* (Brandt *et al.*, 1998; Muoio *et al.*, 2002).

In the research, we used a level of SP supplementation based on the recommended doses for human species and adapted it to rabbits. This SP dosage has not shown any significant effect on the morphological data and some parameters in plasma of the rabbits fed the HF diets, but has shown a significant effect on reducing the level of ROS in plasma.

Although, only a few human studies have been carried out, so far, on the health benefits, SP is already in use in new health care approaches. Further clinical research, using higher levels of SP supplementation will help solidify the benefits of its use.

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REFERENCES

- Alberti, K.G.M.M. and P.Z. Zimmet, 1998. Definition, diagnosis and classification of *diabetes mellitus* and its complications. Part 1: diagnosis and classification of *diabetes mellitus*. Provisional Report of a WHO Consultation. *Diabet. Med.*, 15: 539-553. http://whqlibdoc.who.int/hq/1999/WHO_NCD_NC_S_99.2.pdf.
- AOAC (Association of Official Analytical Chemists), 1990. *Official Methods of Analysis*. 15th Edn. Washington, DC: Association of Official Analytical Chemists. ISBN: 0935584420.
- Bhat, V.B. and K.M. Madyastha, 2000. Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from *Spirulina platensis*: Protection against oxidative damage to DNA. *Biochem. Biophys. Res. Commun.*, 285: 262-266. DOI: 10.1006/bbrc.2001.5195.
- Belay, A., 2002. The potential application of *Spirulina* (*Arthrospira*) as a nutritional and therapeutic supplement in health management. *J. Am. Nutraceut. Assoc.*, 5 (2): 27-48. <http://www.ana-jana.org/Journal/journals/JANAVol52.pdf>.
- Brandt, J.M., F. Djouadi and D.P. Kelly, 1998. Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor alpha. *J. Biol. Chem.*, 273: 23786-23792. DOI: 10.1074/jbc.273.37.23786.
- Broedl, U.C., W. Jin, D. Marchadier, A. Secreto and D.J. Rader, 2005. Tissue-specific expression pattern of human endothelial lipase in transgenic mice. *Atherosclerosis*, 181: 271-274. DOI: 10.1016/j.atherosclerosis.2005.01.027.
- Brousseau, M.E. and J.M. Hoeg, 1999. Transgenic rabbits as models for atherosclerosis research. *J. Lipid Res.*, 40: 365-375. <http://www.jlr.org/cgi/reprint/40/3/365>.
- Caballero, A.E., 2003. Endothelial dysfunction in obesity and insulin resistance: A road to diabetes and heart disease. *Obes. Res.*, 11: 1278-1289. DOI: 10.1038/oby.2003.174.
- Carroll, J.F., T.M. Dwyer, A.W. Grady, G.A. Reinhart, J.P. Montani, K. Cockrell, E.F. Meydrech and H.L. Mizelle, 1996. Hypertension, cardiac hypertrophy and neurohumoral activity in a new animal model of obesity. *Am. J. Physiol. Heart Circ. Physiol.*, 271: H373-H378. <http://ajpheart.physiology.org/cgi/reprint/271/1/H373>.
- Carroll, J.F. and S.C. Tyagi, 2005. Extracellular matrix remodelling in the heart of the homocysteinemic obese rabbit. *Am. J. Hypertens.*, 18: 692-698. DOI: 10.1016/j.amjhyper.2004.11.035.
- Chouinard, P.Y., L. Corneau, A. Sæbø and D.E. Bauman, 1999. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. *J. Dairy Sci.*, 82: 2737-2745. PMID: 20000404646.
- Christie, W.W., 1982. A simple procedure for rapid transmethylolation of glycerolipids and cholesteryl esters. *J. Lipid Res.*, 23: 1072-1075. <http://www.jlr.org/cgi/reprint/23/7/1072?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=1&author1=Christie%2C+WW&andorexactitle=and&andorexacttitleabs=and&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&fdate=10/1/1981&tdate=10/31/1983&resourcetype=HWCIT>.
- Clark, J.M., F.L. Brancati and A.M. Diehl, 2002. Nonalcoholic fatty liver disease. *Gastroenterology*, 122 (6): 1649-1657. DOI: 10.1053/gast.2002.33573.
- Dasgupta, T., S. Banerjee, P.K. Yadav and A.R. Rao, 2001. Chemomodulation of carcinogen metabolising enzymes, antioxidant profiles and skin and forestomach papillomagenesis by *Spirulina platensis*. *Mol. Cell. Biochem.*, 226: 27-38. DOI: 10.1023/A:1012769215383.
- Delbosc, S., E. Palzanis, R. Magous, C. Araiz, J.P. Cristol, G. Cros and J. Azay, 2005. Involvement of oxidative stress and NADPH oxidase activation in the development of cardiovascular complications in a model of insulin resistance, the fructose-fed rat. *Atherosclerosis*, 179 (1): 43-49. DOI: 10.1016/j.atherosclerosis.2004.10.018.
- Eckel, R.H., S.M. Grundy and P.Z. Zimmet, 2005. The metabolic syndrome. *Lancet*, 365: 1415-1428. DOI: 10.1016/S0140-6736(05)66378-7.

- Flier, J.S., 2004. Obesity wars: molecular progress confronts an expanding epidemic. *Cell*, 116: 337-350. DOI: 10.1016/S0092-8674(03)01081-X.
- Gonzalez de Rivera, C., R. Miranda-Zamora, J.C. Diaz-Zagoya and M.A. Juarez-Oropeza, 1993. *Spirulina maxima* prevents induction of fatty liver induced by a fructose-rich diet in the rat: A preliminary report. *Life Sci.*, 53 (1): 57-61. http://www.sciencedirect.com/science?_ob=MIimg&_imagekey=B6T99-475WN86-2FT-1&_cdi=5109&_user=525216&_orig=browse&_coverDate=12%2F31%2F1993&_sk=999469998&view=c&wchp=dGLzVtb-zSkWz&md5=659dcc48314dcc07c54dbd897cb42718&ie=/sdarticle.pdf.
- Halliwell, B. and J.M.C. Gutteridge, 1989. *Free Radicals in biology and Medicine*. Clarendon Press, Oxford, UK. ISBN: 0198552912.
- Hara, A. and N.S. Radin, 1978. Lipid extraction of tissues with a low-toxicity solvent. *Anal. Biochem.*, 90: 420-426. PMID: 19791479896.
- Henrikson, R., 1997. *Earthfood spirulina*. Renore Enterprises, Inc., Kenwood, California., pp: 187. ISBN: 0-9623111-0-3.
- Honjo, T. and N. Inoue, 2006. Antioxidant drugs as the strategy for treatment of metabolic syndrome. *Nippon Rinsho Jpn. J. Clin. Med.*, 64: 660-667.
- Hotamisligil, G.S., N.S. Shargill and B.M. Spiegelman, 1993. Adipose expression of tumor necrosis factor- α : Direct role in obesity-linked insulin resistance. *Science*, 259: 87-91. DOI: 10.1126/science.7678183.
- Hotamisligil, G.S., P. Peraldi, A. Budavari, R. Ellis and M. White, 1996. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF- α -and obesity-induced insulin resistance. Adipose expression of tumor necrosis factor- α : Direct role in obesity-linked insulin resistance. *Science*, 271: 665-668. DOI: 10.1126/science.271.5249.665.
- Ichikawa, T., S. Kitajima, J. Liang, T. Koike, X. Wang, H. Sun, M. Okazaki, M. Morimoto, H. Shikama, T. Watanabe, N. Yamada and J. Fan, 2004. Overexpression of lipoprotein lipase in transgenic rabbits leads to increased small dense LDL in plasma and promotes atherosclerosis. *Lab. Invest.*, 84: 715-726. DOI: 10.1038/labinvest.3700102.
- Iwata, K., T. Inayama and T. Kato, 1987. Effects of *Spirulina platensis* on fructose-induced hyperlipidemia in rats. *J. Jpn. Soc. Nutr. Food Sci.*, 40: 463-467.
- Iwata, K., T. Inayama and T. Kato, 1990. Effects of *Spirulina platensis* on plasma lipoprotein lipase activity in fructose-induced hyperlipidemic rats. *J. Nutr. Sci. Vitaminol.*, 36: 165-171. <http://www.journalarchive.jst.go.jp/jnlpdf.php?cdjournal=jnsv1973&cdvol=36&noissue=2&startpage=165&lang=en&from=jnlto>.
- Jernas, M., J. Palming, K. Sjöholm, E. Jennische, P.A. Svensson, B.G. Gabrielsson, M. Levin, A. Sjögren, M. Rudemo, T.C. Lystig, B. Carlsson, L.M.S. Carlsson and M. Lonn, 2006. Separation of human adipocyte by size: Hypertrophic fat cells display distinct gene expression. *FASEB J.*, 20 (9): 1540-1542. DOI: 10.1096/fj.05-5678fje.
- Kawai, T., T. Ito, K. Ohwada, Y. Mera, M. Matsushita and H. Tomoike, 2006. Hereditary postprandial hypertriglyceridemic rabbit exhibits insulin resistance and central obesity. A novel model of metabolic syndrome. *Arterioscler. Thromb. Vasc. Biol.*, 26: 2752-2757. DOI: 10.1161/01.ATV.0000245808.12493.40.
- Kobayashi, H., T. Nakamura, K. Miyaoka, M. Nishida, T. Funahashi, S. Yamashita and Y. Matsuzawa, 2001. Visceral fat accumulation contributes to insulin resistance, small-sized low density lipoprotein and progression of coronary artery disease in middle-aged non obese Japanese men. *Jpn. Circ. J.*, 65: 193-199. <http://nls.nii.ac.jp/els/110002567018.pdf> id=ART0002821349&type=pdf&lang=en&host=cinii&order_no=&ppv_type=0&lang_sw=&no=1251881592&cp.
- Kljno, T., M. Hozumi, T. Morinobu, T. Murata, Z. Mingci and H. Tamai, 1998. Antioxidant vitamin levels in plasma and low density lipoprotein of obese girls. *Free Radical Res.*, 28: 81-86. DOI: 10.3109/10715769809097878.
- Lissi, E.A., M. Pizzarro, A. Aspee and C. Romay, 2000. Kinetics of phycocyanine bilin groups destruction by peroxy radicals. *Free Rad. Biol. Med.*, 28: 1051-1055. http://www.sciencedirect.com/science?_ob=MIimg&_imagekey=B6T38-40BG7S4-5-1&_cdi=4940&_user=525216&_orig=browse&_coverDate=04%2F30%2F2000&_sk=999719992&view=c&wchp=dGLbVlzSkWA&md5=3a4495ed52449fe985cfff2beefe6243d&ie=/sdarticle.pdf.
- Madsen, L., R.K. Petersen and K. Kristiansen, 2005. Regulation of adipocyte differentiation and function by polyunsaturated fatty acids. *Biochim. Biophys. Acta*, 1740 (2): 266-286. DOI: 10.1016/j.bbadis.2005.03.001.
- Maeda, N., I. Shimomura, K. Kishida, H. Nishizawa and M. Matsuda, 2002. Diet-induced insulin resistance in mice lacking adiponectin/ACRP 30. *Nat. Med.*, 8: 731-737. DOI: 10.1038/nm724.
- Mani, U.V., A. Sadliwala, U.M. Iyer and P.M. Parikh, 2000. The effect of *Spirulina* supplementation on blood haemoglobin levels of anaemic adult girl. *J. Food Sci. Technol.*, 37: 642-644.
- Manoj, G. and S.L. Venkataraman, 1992. Antioxidant Properties of *Spirulina (Spirulina platensis)*. In: Jeeji Bai N. (Ed.). *Spirulina*. ETTA Nat. Symp, MCRC: Madras, India, pp: 48-154.

- Meerarani, P., J.J. Badimon, E. Zias, V. Fuster and P.R. Moreno, 2006. Metabolic syndrome and diabetic atherothrombosis: Implications in vascular complications. *Curr. Mol. Med.*, 6: 501-514. DOI: 10.2174/156652406778018680.
- Miranda, M.S., R.G. Cintra, S.B. Barros and J. Mancini Filho, 1998. Antioxidant activity of the microalga *Spirulina maxima*. *Braz. J. Med. Biol. Res.*, 31:1075-1079. DOI:10.1590/S0100-879X199800080007.
- Muoio, D.M., J.M. Way, C.J. Tanner, D.A. Winegar, S.A. Kliewer, J.A. Houmard, W.E. Kraus and G.L. Dohm, 2002. Peroxisome proliferator-activated receptor- α regulates fatty acid utilization in primary human skeletal muscle cells. *Diabetes*, 51: 901-909. DOI: 10.2337/diabetes.51.4.901.
- Muredach, P., M.P. Reilly and D.J. Rader, 2003. The metabolic syndrome: More than the sum of its parts? *Circulation*, 108: 1546-1551. DOI: 10.1161/01.CIR.0000088846.10655.E0.
- Nakaya, N., Y. Homma and Y. Goto, 1988. Cholesterol lowering effect of Spirulina. *Nutr. Rep. Int.*, 37: 1329-1337. http://grande.nal.usda.gov/ibids/index.php?mode2=detail&origin=ibids_references&throw=423615.
- National Research Council (NRC), 1977. Nutrient Requirements of Domestic Animals, No. 9. Nutrient Requirements of Rabbits. 2nd Rev. Edn. National Academy of Sciences-National Research Council, Washington, DC. <http://www.nap.edu/openbook.php?isbn=0309026075>.
- Ravindranath, V., 1994. Animal models and molecular markers for cerebral ischemia-reperfusion injury in brain. *Method Enzymol.*, 233: 610-619. http://www.sciencedirect.com/science?_ob=MIimg&_imagekey=B7CV2-4B3T1CX-H5-1&_cdi=18066&_user=525216&_orig=browse&_coverDate=12%2F31%2F1994&_sk=997669999&view=c&wchp=dGLbVzz-zSkWA&md5=ff441a156429f7cca3ec2be4a05fec21&ie=/sdarticle.pdf.
- Reaven, G.M., 1988. Role of insulin resistance in human diseases. *Diabetes*, 37: 1595-1607. DOI: 10.2337/diabetes.37.12.1595.
- Richmond, A., 1992. Mass Culture of Cyanobacteria. In: Man, N.H. and N.G. Carr (Eds.). *Biotechnology Handbooks (Photosynthetic Prokaryotes)*. Springer-Verlag New York, LLC, pp: 181-210. ISBN: 13: 9780306438790.
- Roberts, C.K., J. Barnard, R.K. Sindhu, M. Jurczak, A. Ehdaie and N.D. Vaziri, 2006. Oxidative stress and dysregulation of NADPH oxidase and antioxidant enzymes in diet-induced metabolic syndrome. *Metabolism*, 55: 928-934. DOI: 10.1016/j.metabol.2006.02.022.
- Robertson, J.B. and P.J. Van Soest, 1981. The Detergent System of Analysis. In: James, W.P.T. and O. Theander (Eds.). *The Analysis of Dietary Fibre in Food*. Marcel Dekker, N.Y., USA, Chapter, 9: 123-158. DOI: 10.1016/S0031-9384(00)00286-9.
- Rodriguez-Hernandez, A., J.L. Blè-Castillo, M.A. Juárez-Oropeza and J.C. Díaz-Zagoya, 2001. *Spirulina maxima* prevents fatty liver formation in CD-1 male and female mice with experimental diabetes. *Life Sci.*, 69: 1029-1037. PII: S0024-3205(01)01185-7.
- Skurk, T. and H. Hauner, 2004. Obesity and impaired fibrinolysis: Role of adipose production of plasminogen activator inhibitor-1. *Int. J. Obesity*, 28: 1357-1364. DOI: 10.1038/sj.ijo.0802778.
- Stienstra, R., C. Duval, M. Muller and S. Kersten, 2007. PPARs, Obesity and inflammation. *PPAR Res.*, 1: 1-10. DOI: 10.1155/2007/95974.
- Takai, Y., Y. Hossayamada and T. Kato, 1991. Effect of water soluble and water insoluble fractions of Spirulina over serum lipids and glucose resistance of rats. *J. Jpn. Soc. Nutr. Food Sci.*, 44: 273-277.
- Ulbricht, T.L. and D.A.T. Southgate, 1991. Coronary heart disease: seven dietary factors. *Lancet*, 338: 985-992. DOI: 10.1016/0140-6736(91)91846-M.
- Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, 74: 3583-3591. <http://jds.fass.org/cgi/reprint/74/10/3583?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&andorexactitle=and&andorexactitleabs=and&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&firstpage=3583&resourceType=HWCIT>.
- Vaziri, N.D., 2004. Roles of oxidative stress and antioxidant therapy in chronic renal disease and hypertension. *Curr. Opin. Nephrol. Hy.*, 13: 93-99. Accession Number: 00041552-200401000-00013.
- Wautier, M.P., O. Chappey, S. Corda, D.M. Stern, A.M. Schmidt and J.L. Wautier, 2001. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. *Am. J. Physiol. Endocrinol. Metab.*, 280: 685-694. <http://ajpendo.physiology.org/cgi/reprint/280/5/E685?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&author1=Wautier&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&resourceType=HWCIT>.
- Zhao, S., Y. Chu, C. Zhang, Y. Lin, K. Xu, P. Yang, J. Fan and E. Liu, 2008. Diet-induced central obesity and insulin resistance in rabbits. *J. Anim. Physiol. An. N.*, 92: 105-111. DOI: 10.1111/j.1439-0396.2007.00723.x.