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Effects of fish oil on hypertension, plasma lipids, and tumor necrosis factor-α in rats with sucrose-induced metabolic syndrome

Alfonso Alexander Aguilera, Guillermo Hernández Díaz, Martín Lara Barcelata, Ofelia Angulo Guerrero, Rosa M. Oliart Ros*

Instituto Tecnológico de Veracruz, UNIDA, Apdo. Postal 1420, Veracruz, Ver. México

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Abstract

Dietary fish oil rich in (n-3) fatty acids plays an important role in reducing abnormalities associated with the metabolic syndrome and mortality from coronary heart disease. We investigated the effects of dietary fish oil on the metabolic syndrome in a high-sucrose–fed rat model. The model was achieved by the administration of 30% sucrose in drinking water in male Wistar rats during 21 weeks. After the metabolic syndrome rat model was established, fish oil was administered during 6 weeks. The metabolic syndrome rats showed significant increases in body weight, systolic blood pressure, serum insulin, total lipids, triacylglycerols, cholesterol, free fatty acids, LDL, total proteins, albumin, and serum tumor necrosis factor–α (TNF-α). They also presented abdominal and epididymal fat accumulation and fatty liver. After fish oil diet administration, metabolic syndrome rats had a significant reduction in blood pressure, serum insulin, triacylglycerols, cholesterol, free fatty acids, and total lipids, but no change was observed in TNF-α concentration or fat accumulation. In conclusion, fish oil reversed the alterations on metabolic parameters and blood pressure exerted by sucrose administration, although it had no effect on TNF-α production and adiposity. This confirms the theory that the molecular etiology of the metabolic syndrome is multifactorial, as is the effect of n-3 polyunsaturated fatty acids (PUFAs) upon it, having complex and multifaceted actions.

Keywords: Fish oil; Metabolic syndrome; Sucrose-induced model; TNF-α

1. Introduction

In 1988, Gerald Reaven introduced the concept of "syndrome X" for the clustering of cardiovascular risk factors such as hypertension, glucose intolerance, high triacylglycerols, and low HDL cholesterol concentrations found in individuals prone to develop cardiovascular diseases (CVD), and proposed that the common denominator of the syndrome was resistance to the action of insulin [1]; other metabolic abnormalities have been associated with this syndrome, including obesity, microalbuminuria, alterations in fibrinolysis, and coagulation [2,3]. The syndrome has also been called insulin resistance syndrome, cardiovascular syndrome, and more recently metabolic syndrome. Recent studies have suggested that inflammatory mediators such as tumor necrosis factor-α (TNF-α) and interleukins (ILs) play a central role in the development of cardiovascular diseases. Elevated plasma levels of TNF-α have been found to be associated with obesity, insulin resistance, hypertriglyceridemia, and glucose intolerance [4].

CVDs have become the leading cause of morbidity and mortality in western countries [5], totaling 22.43% of all deaths in Mexico in 2002 [6]. CVDs are one of the significant diet-related health problems; there are a number of epidemiological studies supporting the dietary regulation of each of the metabolic risk factors of metabolic syndrome [7–10], and there is a great deal of interest in how dietary fat composition influences the development of this syndrome. Long-chain (n-3) polyunsaturated fatty acids have attracted considerable attention for the last few years, as dietary fish oil rich in EPA (20:5 n-3) and DHA (22:6 n-3) fatty acids plays an important role in reducing hypertriglyceridemia and seems to lower mortality from coronary heart disease [11–13]. It is important to note, however, that discrepancies exist regarding differences in the effects on metabolic syndrome parameters after fish oil or n-3 fatty acid administration, as well as in the mechanisms proposed to explain such effects.

* Corresponding author. Tel.: 52 (229) 9 34 57 01 ext 112; fax: 52 (229) 9 34 57 01 ext 201.

E-mail address: roliart@itver.edu.mx (R.M. Oliart Ros).

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An experimental model that resembles metabolic syndrome can be induced in rats by the administration of high-fructose or high-sucrose diets [14–16]. In our laboratory this model has been used, exhibiting insulin resistance, hyperinsulinemia, impaired glucose tolerance, hypertriglyceridemia, and mild hypertension [13].

The purpose of this study was to determine the effect of a fish oil diet on blood pressure, insulin concentration, lipid metabolic parameters, and serum concentration of TNF-α in a metabolic syndrome–induced rat model.

2. Methods and materials

2.1. Materials

All chemicals were of analytical grade, obtained from Sigma Chemical Co. (St. Louis, MO), Bayer (Mexico City, Mexico), and J.T. Baker (Mexico City, Mexico). Dietary components were purchased from Harlan Teklad Inc. (Madison, WI). Insulin radioimmunoassay kit was obtained from Diagnostic Products Corporation (Los Angeles, CA). The nonesterified fatty acids kit was bought from Wako Chemical (Neuss, Germany) and rat TNF-α kit was purchased from R&D System Inc. (Minneapolis, MN).

2.2. Sucrose-induced metabolic syndrome model

A total of 44 weaning male Wistar rats (National Institute of Medical Sciences and Nutrition "Salvador Zubiran," Mexico), 21 days of age, were individually housed and maintained in a 12-h light/dark cycle at 25°C. Animal maintenance and handling were in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" [17]. Animals were divided into two groups: the control group (C; $n = 12$), which received a standard diet (Lab Diet 2001, Harlan Teklad Inc.); and the metabolic syndrome group (MS; $n = 32$), which received the standard diet plus 30% sucrose in drinking water [18]. After animals were fed *ad libitum* during 21 weeks (Fig. 1), their body weight, blood pressure, insulin, glucose, triacylglycerols, free fatty acids, cholesterol, TNF-α, HDL, LDL, and total lipids were measured. Other parameters as creatinine, uric acid, AST, ALT, total proteins, and albumin were also determined. A total of 19 animals were used to obtain organs for further analysis.

2.3. Experimental diet

Animals with metabolic syndrome (MS), obtained as described above, were divided into two groups: a corn–canola oil diet (CC-MS) group, in which the lipid source consisted of a 7.5% corn and canola oil mixture (Patrona, from the local market), and a fish oil diet (FO-MS) group, using 7.5% of fish oil (sardine oil, Sonora University, Mexico) as the sole source of lipids (11% eicosapentaenoic acid (EPA, 20:5 n-3), 7% docosahexaenoic acid (DHA 22:6 n-3) (Tables 1 and 2). These two groups received diets and drinking water with 30% sucrose during 6 weeks (Fig. 1). The control group (CC) received the corn–canola oil diet and no sucrose in the drinking water. Diets were prepared using butylated hydroxy toluene (BHT) at 0.02% as an antioxidant and stored under refrigeration until the end of the study.

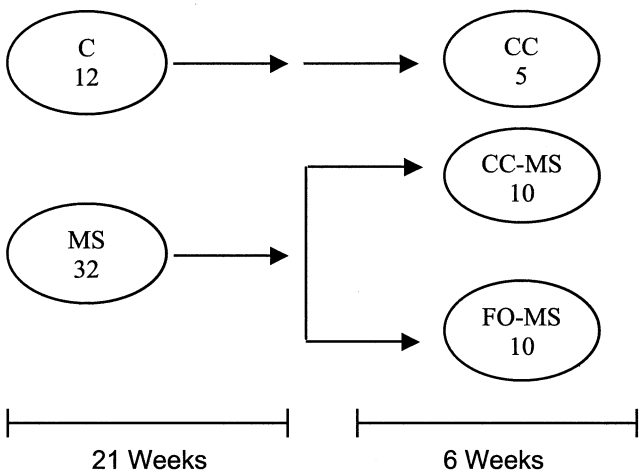

Fig. 1. Dietary protocol. Experimental models: C = chow diet plus plain water; MS = chow diet plus 30% sucrose in drinking water. Experimental diets: CC = corn–canola oil diet plus water; CC-MS = corn–canola oil diet plus 30% sucrose in drinking water; FO-MS = fish oil diet plus 30% sucrose in drinking water.

At the end of the experimental diet period, fasted animals were killed by decapitation (without anesthesia to avoid interference with insulin measurements) and the metabolic syndrome parameters were determined.

Table 1
Composition of diets administered to rats

Ingredient	Corn–Canola Oil Diet (g)	Fish Oil Diet (g)
Casein	440	440
DL-methionine	3.2	3.2
Cellulose	40	40
Starch	658	658
Vitamin mix*	20	20
Mineral mix†	80	80
Corn oil	50	—
Canola oil	50	—
Fish oil	—	100

* Vitamin mix (Teklad 40060): p-aminobenzoic acid, ascorbic acid, biotin, vitamin B_{12}, calcium pantothenate, choline dihydrogen citrate, folic acid, inositol, menadione, niacin, pyridoxine HCL, riboflavin, thiamin HCL, dry vitamin A palmitate, dry vitamin D_3, dry vitamin E acetate, corn starch.

† Mineral mix (Teklad AIN-76 170915): $CaHPO_4$, NaCl, potassium citrate, K_2SO_4, MgO, manganous carbonate, ferric citrate, zinc carbonate, cupric carbonate, potassium sulfate, sucrose.

Table 2
Fatty acid composition of diets administered to rats

Fatty Acid	Chow Diet (%)	Corn–canola Diet (%)	Fish Oil Diet (%)
Saturated			
10:0	nd	0.05	nd
12:0	nd	nd	0.04
14:0	2.50	0.55	7.28
16:0	30.0	12.80	31.46
18:0	5.00	nd	9.04
20:0	nd	nd	0.73
22:0	nd	0.32	0.32
Total	37.50	12.80	48.87
Monounsaturated			
14:1	0.50	nd	0.26
16:1	nd	nd	7.78
18:1	55.0	50.70	19.11
20:1	3.00	nd	nd
24:1	nd	nd	nd
Total	58.50	50.70	27.15
Polyunsaturated			
n-6			
18:2	9.50	32.80	0.48
20:4	nd	nd	0.36
Total	9.50	32.80	0.84
n-3			
18:3	2.50	3.5	2.85
18:4	nd	nd	nd
20:5	nd	nd	20.00
22:6	nd	nd	13.00
Total	2.50	3.5	35.85
n-6/n-3	3.8	9.30	0.02

Values are expressed as percentage of total fatty acids.
nd = not detected.

2.4. Blood samples

At the end of each treatment period, blood samples were carefully collected to avoid hemolysis on 18-hour–fasted animals. The blood was centrifuged at 1086 × *g* for 10 min and serum was kept at –20°C until analysis.

2.5. Blood pressure measurement

Systolic blood pressure was estimated by a tail-cuff method (IITC noninvasive blood pressure system, model 29; Life Science Instruments, Woodland Hills, CA) in conscious animals. The reported blood pressure value is the mean of five systolic measurements.

2.6. Analytical methods

Serum glucose concentration was measured by the glucose oxidase method [19]. Serum insulin concentration was determined by a commercial double-antibody solid-phase radioimmunoassay (Coat-A-Count, DPC). Serum FFAs were determined from fresh frozen samples by an enzymatic method (NEFA-C test, Wako Chemicals GmbH). Total cholesterol was measured using enzymatic reagents [20]. Serum HDL was determined after precipitation of LDL and very–low density lipoprotein (VLDL) cholesterol with phosphotungstic acid in the presence of Mg^{+} ions [21]. Triacylglycerols, total lipids, and uric acid were measured by means of an enzymatic technique according to the manufacturer's instructions. Creatinine, uric acid, total proteins, albumin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined by colorimetric or enzymatic methods (Bayer Diagnostics, Tarrytown, NY, USA) in a RA 10,000 Autoanalyzer (Bayer Diagnostics, Tarrytown, NY, USA).

Finally, serum TNF-α levels were measured using an ELISA assay described for rat TNF-α (Quantikine, R&D Systems). This is a newly developed, sensitive sandwich ELISA with a low detection limit (0.5 pg/mL).

2.7. Lipids extraction and fatty acid composition determination

Lipids were extracted from diets according to Folch et al. [22]. Fatty acids were converted to methyl esters by H_2S-catalyzed transmethylation [23] and analyzed by gas chromatography using a Hewlett Packard Gas Chromatograph, model 6890 (Andover, MA) equipped with a Carbowax capillary column and a flame ionization detector. Injection and detector temperatures were 250°C, nitrogen was the carrier gas and the column temperature was programmed to rise from 100 to 210°C at a rate of 2°C/min. Fatty acid methyl esters were identified by comparison with fatty acid standards (Sigma Chemical Co.).

2.8. Data analysis

Data are presented as the mean ± SD. Statistical significance was determined by analysis of variance procedures, and a Tukey multiple range test was used for mean comparison ($P < 0.05$).

3. Results

3.1. Experimental model

The metabolic syndrome model was achieved by the administration of 30% sucrose in drinking water in male Wistar rats during 21 weeks. Figure 2 shows a significant difference in the evolution of body weight in rats with (MS group) and without (C group) sugar ingestion. At 10 weeks, a moderate increase in body weight in MS group was observed ($P < 0.01$), but at 21 weeks, body weight in MS group was significantly higher ($P < 0.001$) than in C group.

Table 3 shows the differences in liquid consumption as well as food and caloric intake between the two groups ($P < 0.001$) at the end of the treatment period (last 6 weeks). Ingestion of a high amount of sucrose led to a significant increase in abdominal (4-fold, $P < 0.001$) and epididymal

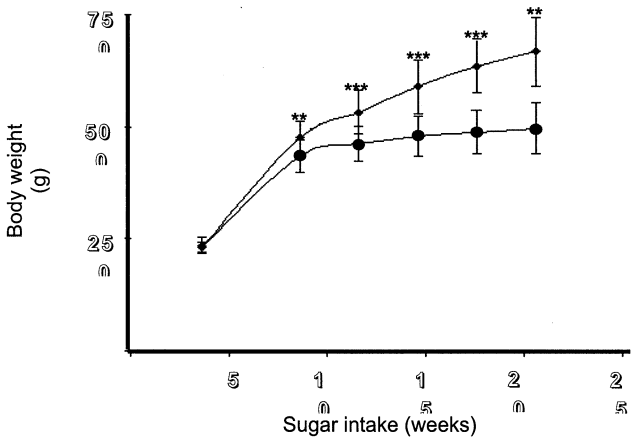

Fig. 2. Body weight evolution of sucrose-fed and control rats. Filled circles indicate controls (C). Filled diamonds indicate sugar-fed rats (MS).**$P < 0.01$; ***$P < 0.001$.

(3-fold, $P < 0.001$) fat accumulation, and an increase in liver weight (1.6-fold, $P < 0.01$). The MS group rats also showed an increase in systolic blood pressure values (1.2-fold, $P < 0.001$), triacylglycerols (2-fold, $P < 0.001$), nonesterified fatty acids (2-fold, $P < 0.001$), insulin (1.5-fold, $P < 0.01$), cholesterol (1.4-fold, $P < 0.01$), total lipids (1.1-fold, $P < 0.001$), total proteins (1.9-fold, $P < 0.01$), albumin (1.3-fold, $P < 0.001$), and the proportion of AST/ALT found was <1 (Table 4). No difference was observed in LDL, glucose, uric acid, and creatinine levels. Lower concentrations of HDL were found ($P < 0.05$) in MS group rats (Table 4).

As shown in Fig. 3, the TNF-α serum concentration was significantly higher in sugar-fed rats (49.91 ± 1.29 pg/mL) than in the control group (37.77 ± 0.57 pg/mL) ($P < 0.001$). To the best of our knowledge, this is the first time that increases in TNF-α serum levels in sucrose-induced metabolic syndrome model have been reported.

Table 3
Liquid consumption, food and caloric intake, and fat and liver weight in sucrose-fed (MS group) and control (C group) rats

Parameter	C Group	MS Group
Liquid consumption (mL/day)	48.42 ± 3.22	60.13 ± 3.04*
Liquid consumption (mL/day/100 g bw)	11.02 ± 0.71	9.57 ± 1.26
Equivalent in Kcal in drinking water	0	11.48 ± 1.51*
Food consumption (g/day)	25.35 ± 1.24	12.34 ± 1.32*
Food consumption (g/day/100 g bw)	5.77 ± 0.28	2.03 ± 0.23*
Equivalent in Kcal in food	7.64 ± 0.38	2.70 ± 0.30*
Total Kcal/day/100 g bw	7.64 ± 0.37	14.06 ± 1.35*
Abdominal fat weight (g)	6.52 ± 2.70	27.72 ± 7.81*
Epididymal fat weight (g)	5.71 ± 3.15	16.50 ± 4.42*
Liver weight (g)	10.57 ± 2.82	17.26 ± 2.71*

Values are mean ± SD (C, $n = 5$; SX, $n = 10$).
* $P < 0.001$.
bw = body weight.

Table 4
Blood pressure and serum parameters in control (C) and sucrose-fed (MS) rats

Parameter	C Group	MS Group
Systolic blood pressure (mm Hg)	133.30 ± 10.12	167.22 ± 9.18*
Insulin (μUI/mL)	8.64 ± 2.10	13.60 ± 3.80†
Glucose (mmol/L)	4.27 ± 0.84	4.74 ± 1.18
Triacylglycerols (mmol/L)	4.34 ± 0.42	8.92 ± 2.01*
Free fatty acid (mmol/L)	0.51 ± 0.11	1.05 ± 0.09*
Cholesterol (mmol/L)	3.25 ± 0.88	4.58 ± 0.90†
HDL (mmol/L)	3.22 ± 0.63	2.52 ± 0.49‡
LDL (mmol/L)	3.55 ± 0.27	5.16 ± 0.32*
Total lipids (mmol/L)	46.64 ± 0.42	54.94 ± 3.93*
Total proteins (mmol/L)	0.21 ± 0.10	0.41 ± 0.12†
Albumin (mmol/L)	0.17 ± 0.02	0.23 ± 0.01†
Uric acid (mmol/L)	0.08 ± 0.03	0.10 ± 0.02
Creatinine (mmol/L)	0.03 ± 0.01	0.04 ± 0.01
AST (U/L)	39.80 ± 4.08	62.33 ± 6.70*
ALT (U/L)	35.40 ± 7.40	305.17 ± 10.80*
AST/ALT	1.12	0.20

Values are mean ± SD. C group, $n = 12$; MS group, $n = 32$.
* $P < 0.001$.
† $P < 0.01$.
‡ $P < 0.05$.

3.2. Effects of fish oil

After the sucrose-induced metabolic syndrome rat model was established, the effects of dietary fish oil were analyzed. Table 5 shows no difference in liquid consumption, food and caloric intake, body weight, or abdominal and epididymal fat weight between CC-MS and FO-MS groups after 6 weeks of experimental diet administration.

Blood pressure in animals receiving the corn–canola oil diet plus sucrose in drinking water (CC-MS) was significantly higher (1.5-fold, $P < 0.001$) than in animals under nonsucrose corn–canola oil (CC) diet (Table 6). FO-MS group presented blood pressure values similar to those found in the CC group, being 60% ($P < 0.001$) lower than in the CC-MS group.

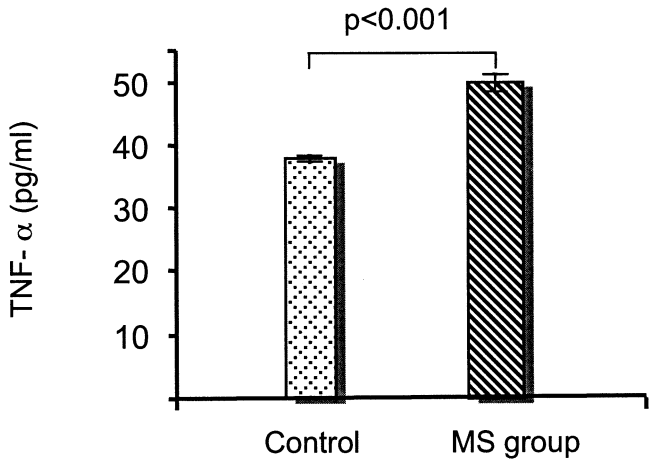

Fig. 3. TNF-α serum concentrations in control (C) and sucrose-fed (MS) rats. Values are mean ± SD.

Table 5
Body weight, abdominal and epididymal fat weight, liquid consumption, and food and caloric intake in rats fed experimental diets during 6 weeks

Variable	CC Group	CC-MS Group	FO-MS Group
Body weight initial (g)	441.48 ± 45.05[a]	552.75 ± 57.41[b]	530.67 ± 72.81[b]
Body weight final (g)	463.31 ± 52.18[a]	610.67 ± 66.88[b]	597.02 ± 81.89[b]
Abdominal fat weight (g)	8.09 ± 4.39[a]	24.12 ± 9.02[b]	24.23 ± 9.70[b]
Epididymal fat weight (g)	7.09 ± 2.38[a]	14.83 ± 4.84[b]	14.69 ± 4.70[b]
Liquid consumption (mL/day)	40.56 ± 5.44[a]	51.92 ± 4.26[b]	50.05 ± 5.33[b]
Liquid consumption (mL/day/100 g bw)	8.71 ± 1.17[a]	8.68 ± 0.64[a]	8.64 ± 1.07[a]
Equivalent in Kcal in drinking water	0[a]	10.43 ± 0.76[b]	10.37 ± 1.28[b]
Food consumption (g/day)	18.86 ± 1.68[a]	11.61 ± 2.36[b]	10.09 ± 2.73[b]
Food consumption (g/day/100 g bw)	3.95 ± 0.48[a]	1.83 ± 0.49[b]	1.70 ± 0.25[b]
Equivalent in Kcal in food	15.58 ± 1.90[a]	7.23 ± 1.94[b]	6.68 ± 1.03[b]
Total Kcal/day/100 g bw	15.58 ± 1.90[a]	17.66 ± 2.64[a]	17.05 ± 1.86[a]

Values are means ± SD.
Corn–canola diet (CC group $n = 5$); corn–canola diet plus 30% sucrose in drinking water (CC-MS group, $n = 10$); fish oil diet plus 30% sucrose in drinking water (FO-MS group, $n = 10$).
$P < 0.01$ with respect to CC group.
Different superscript letters represent differences between groups.

Findings were similar for serum insulin concentrations. The CC-MS group showed a significant increase in insulin concentrations (2.8-fold, $P < 0.001$) with respect to CC rats. Insulin concentrations in the FO-MS group were similar to those found in CC group, but significantly lower (150%, $P < 0.001$) than those in CC-MS group. FO-MS rats showed significantly lower concentrations of serum triacylglycerols (98%, $P < 0.001$), cholesterol (106%, $P < 0.001$), total lipids (84%, $P < 0.001$), and free fatty acids (200%, $P < 0.001$) with respect to the CC-MS group but similar to those found in the CC group. Serum concentration of glucose in the FO-MS group remained similar to CC rats, and LDL was lower (40% $P < 0.01$) with respect the CC-MS group. Finally, fish oil did not modify the TNF-α serum levels, which remained statistically higher with respect to the CC group.

4. Discussion

The purpose of this study was to investigate the effect of fish oil on hypertension, serum parameters, and TNF-α in a metabolic syndrome rat model obtained by means of sucrose administration in drinking water (30%). A group of rats with organic abnormalities including hypertension, obesity, abdominal and epididymal fat accumulation, and serum elevation of total lipids, triacylglycerols, LDL, total cholesterol, insulin, and free fatty acids was developed (MS group) after 21 weeks of sucrose administration. Our results are similar to those reported previously where the administration of fructose- or sucrose-rich diets to experimental animals caused comparable degrees of hyperinsulinemia, hypertriglyceridemia, insulin resistance, and blood pressure elevation [13-16]. Additionally, other biochemical parameters related to this syndrome were also studied (namely AST, ALT, total proteins, albumin, creatinine, uric acid, and TNF-α), which were also found to be elevated in sucrose-fed rats.

It has been reported that high concentrations of dietary sucrose or fructose induce hypertension in animals [13,18,24], and that the magnitude of induced pathology depends on the animal strain, the type of carbohydrate ingested, and the length of the study [25]. In our work, administration of 30% sucrose in drinking water to weaning male Wistar rats during 21 weeks caused a significant increase in blood pressure. In that respect, Pérez et al. [26] have suggested that an early alteration in the proportion of plasma arachidonic acid in sugar-fed rats can be considered as a marker of hypertension development because of the concomitant increase in the synthesis of vasoconstrictive ecosanoids.

The body composition was examined in detail and a significant increase in abdominal adipose tissue was observed in sucrose-fed rats (Table 3), in accordance with findings by Toida et al. [27]. Abdominal fat accumulation has been associated with hyperinsulinemia, hyperlipidemia, and hypertension [28]; however, any direct implication of abdominal fat increase in hypertension development has not been clearly demonstrated and needs further investigation.

The increases in liver size and weight, as well as the fatty liver observed in sucrose-fed rats could be related to the development of nonalcoholic steatosis induced by high sugar ingestion, which has recently been associated with obesity, hyperlipidemia, and diabetes [29]. Additionally, we found high serum concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as an ALT/AST ratio of < 1, confirming hepatic tissue pathology [30]. We also observed an increase in serum free fatty acids, with a concomitant rise in total proteins and albumin serum concentrations. Sucrose-fed rats did not show renal damage or gout development, inasmuch as creatinine and uric acid serum levels were not modified.

Table 6
Blood pressure and serum parameters in rats fed with experimental diets during 6 weeks

Parameter	CC Group	CC-MS Group	FO-MS Group
Blood pressure (mm Hg)	100.53 ± 22.29[a]	153.77 ± 14.63[b]	94.20 ± 17.82[a]
FNT-α (pg/mL)	38.70 ± 0.87[a]	46.61 ± 2.93[b]	47.17 ± 1.94[b]
Insulin (μUI/mL)	4.16 ± 0.59[a]	11.79 ± 3.50[b]	4.71 ± 3.55[a]
Glucose (mmol/L)	5.51 ± 0.69[a]	6.02 ± 1.78[a]	5.19 ± 1.28[a]
Fatty free acid (mmol/L)	0.51 ± 0.17[a]	0.90 ± 0.10[b]	0.30 ± 0.22[a]
Triacylglycerols (mmol/L)	3.95 ± 2.17[a]	9.16 ± 0.93[b]	4.83 ± 1.07[a]
Cholesterol (mmol/L)	5.06 ± 0.62[a]	7.55 ± 0.83[b]	3.36 ± 0.60[c]
LDL (mmol/L)	4.71 ± 0.46[a]	5.60 ± 1.31[b]	3.35 ± 0.56[c]
Cholesterol/LDL	1.07[a]	1.34[b]	1.09[a]
Total lipids (mmol/L)	39.08 ± 5.48[a]	63.02 ± 10.17[b]	34.16 ± 6.95[a]

Values are mean ± SD.

Corn–canola diet (CC group, $n = 5$); corn–canola diet plus 30% sucrose in drinking water (CC-MS group, $n = 10$); fish oil diet plus 30% sucrose in drinking water (FO-MS group, $n = 10$).

$P < 0.01$ with respect CC group.

Different superscript letters represent differences between groups.

It has been suggested that TNF-α plays a central role in the development of the metabolic syndrome [31]. In recent studies, increased expression of TNF-α has been associated with insulin resistance, obesity, hypertriglyceridemia, and glucose intolerance [4,31], suggesting that TNF-α interferes with insulin action by altering the catalytic activity of the insulin receptor. Consequently, it is important to include the measurement of TNF-α serum levels in studies regarding metabolic syndrome and its treatment. In this study, we found a significant increase in TNF-α serum levels after sucrose administration, together with an increase in body weight, serum free fatty acid levels, and visceral fat accumulation.

Once the metabolic syndrome rat model was obtained, we analyzed the effect of fish oil administration on the parameters that were found to be altered after sucrose administration.

The FO diet, high in n-3 polyunsaturated fatty acids (PUFA) provoked a significant reduction in the parameters that were found to be significantly elevated after sucrose feeding, returning them to concentrations similar to those found in control rats, except for TNF-α serum levels.

Fish oil, and in particular long chain n-3 PUFA, have been shown to reduce blood pressure in normotensive [32–34] and hypertensive subjects and experimental animals [35–44], although some studies have failed to demonstrate any effect [45–50]. The detailed mechanisms mediating this antihypertensive effect are unknown, but they have been attributed to a shift in eicosanoid production away from the two-series prostaglandins derived from arachidonic acid, which are potent vasoconstrictors [8,51]. Evidence that links insulin resistance and hyperinsulinemia to the development of hypertension shows that manipulations aimed at creating a state of insulin resistance in experimental animals led to elevated blood pressure. Furthermore, interventions such as dietary weight loss manipulations, physical training, or drug administration that enhance insulin sensitivity and lower plasma insulin concentrations also decrease blood pressure [52,53]. In our study, FO feeding caused a significant reduction in serum insulin concentrations in rats, so this reduction could in part be responsible for the lowering effect found on blood pressure.

The reduction in serum triacylglycerols concentration by fish oil, as demonstrated by our results and those of others [54,55], as well as the reduction in free fatty acids and total lipids serum levels might play an important role in increasing insulin action. A mechanism based on fuel switching with reduced fatty acid availability and increased glucose utilization, the glucose–fatty acid cycle reported by Randle et al. [56], could be involved in the fish oil-induced increase in insulin action, as suggested by Storlein et al. [57].

The effect of n-3 PUFA on TNF-α concentration is controversial. Suppressive effects [12,58–62] as well as stimulatory effects [63,64] of fish oil on TNF-α production have been reported, although some studies report no effect [65–69]. These differences seem to be related to the doses of fish oil administered, the balance between n-6 and n-3 fatty acids, the cell or animal model used, and the inherent level of cytokine production. In our study, serum concentration of TNF-α was not significantly modified by dietary fish oil. However, it has been reported that the amount of TNF-α mRNA and protein correlates positively with adiposity and adipocyte cell volume [4], and that agrees with the fact that abdominal and epididymal fat weight in our rats were not modified either after fish oil administration.

In conclusion, we obtained an animal model for the metabolic syndrome by means of sucrose administration in drinking water (30%), which was characterized by hypertension, metabolic abnormalities, obesity, abdominal and epididymal fat augmentation, hepatomegalia, and TNF-α concentration increases. Fish oil reverted the alterations on metabolic parameters and blood pressure exerted by sucrose administration, although it had no effect on TNF-α production and adiposity. This confirms that the molecular etiology of the metabolic syndrome is multifactorial, and so is the

effect of n-3 PUFAs on it, having complex and multifaceted actions.

Acknowledgments

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