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Dietary fatty acids effects on sucrose-induced cardiovascular syndrome in rats

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Abstract

Cardiovascular disease is one of the leading causes of morbidity and mortality in Mexico. We investigated the effects of omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids on the metabolic syndrome associated with cardiovascular disease in a high-sucrose-fed rat model. The metabolic syndrome-induced rats showed a significant increase in systolic blood pressure, serum insulin, nonfasting serum triglyceride and serum cholesterol levels. Experimental high-sucrose-fed animals received either a n-3 or n-6 enriched diet or a control diet during 6 weeks. Animals fed the n-3 enriched diet had a significant reduction in blood pressure and serum insulin and triglyceride levels. Serum triglyceride levels were also significantly reduced in the n-6-rich diet animals.

Keywords: Polyunsaturated fatty acids; Cardiovascular diseases; Hypertension; Insulin

1. Introduction

Cardiovascular diseases (CVD) are one of the most significant diet-related health problems, representing a major cause of premature death in Western countries. In Mexico, the incidence and prevalence of CVD have increased over the past three decades and CVD have become the leading cause of morbidity and mortality in the country [1]. In 1997, CVD represented the principal cause of deaths, totaling 25% of all deaths in Mexico [2].

The metabolic disorders found in individuals prone to develop CVD, known as metabolic cardiovascular syndrome (CVS), X syndrome or insulin resistance syndrome [3,4], comprise most or all of the following: overweight or obesity with atherogenic triglyceride-rich lipoproteins, low high density lipoprotein (HDL) cholesterol and small dense low density lipoprotein (LDL), hypertension, hypertriglyceridemia, insulin resistance and glucose intolerance. Each component of the syndrome is closely related to each other, creating a reverberating loop such that a worsening of any one component will lead to deterioration of all the others.

There are a number of epidemiological studies supporting the dietary regulation of each of the metabolic risk factors of CVS [5–8]. Early in the 1970s, Bang et al. [9] reported a substantially lower incidence of coronary heart disease in Greenland Eskimos compared with Danes or other Western populations. It has now become evident that polyunsaturated fatty acids (PUFA) may have a role in CVD prevention.

An experimental model that resembles CVS can be induced in rats by administration of high-sucrose or high-fructose diets [10–12]. This model exhibits insulin resistance, hyperinsulinemia, hypertrigliceridemia, impaired glucose tolerance and mild hypertension.

The purpose of this study was to investigate the effects of diets rich in PUFA (n-3 or n-6) on blood pressure, insulin levels and lipid metabolic parameters in a CVS-induced rat model.

2. Materials and methods

2.1. Materials

All chemicals were of analytical grade, obtained from Sigma Chemical Co. (St. Louis, MO, USA), Merck (Mexico City, Mexico), and JT Baker (Mexico City, Mexico). Di-

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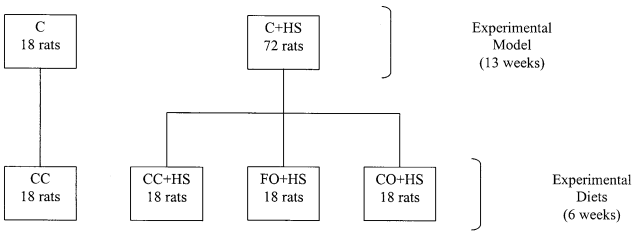

Figure 1. Diet Protocol. Experimental model: C, chow diet plus plain water; C + HS, chow diet plus 30% sucrose in drinking water. Experimental diets: CC, corn-canola diet plus plain water; CC + HS, corn-canola diet plus 30% sucrose in drinking water; FO + HS, fish oil diet plus 30% sucrose in drinking water; CO + HS, corn oil diet plus 30% sucrose in drinking water.

etary components were purchased from Harlan Teklad Inc. (Madison, WI, USA). Insulin radioimmunoassay kit was obtained from Diagnostic Products Corporation (Los Angeles, CA, USA).

2.2. Experimental model

Ninety male Wistar rats were individually housed and maintained in a 12-h light/dark cycle at 25°C. Animal maintenance and handling were in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* [13]. Weaning animals (21 days of age) were divided in two groups: the control group, which received a standard chow (Purina) diet (C; $n = 18$); and the high-sucrose group, which received the chow diet plus 30% sucrose in drinking water (C + HS; $n = 72$) [14], ad libitum, during 13 weeks (Fig. 1). Body weight and blood pressure were measured every 2 weeks.

2.3. Experimental diets

Animals presenting serum elevation of glucose, insulin, triglyceride and cholesterol levels after being under the above diet for 13 weeks were divided into four groups receiving one of the following diets for 6 more weeks (Fig. 1): the C group received a diet containing 7.5% corn and canola oil mixture ("Patrona," from the local market) as lipid source (CC group). This group received drinking water without sucrose. Fifty-four C + HS rats were randomly assigned to three different semisynthetic diets (see Tables 1 and 2: a corn-canola oil (CC) diet (CC + HS group), with the lipid source consisting in 7.5% corn and canola oil mixture ("Patrona," from the local market); a fish oil (FO) diet (FO + HS group), using 7.5% FO (Menhaden oil, Zapata Inc.) as the sole lipid source; and a corn oil (CO) diet (CO + HS group), using 7.5% CO ("Patrona," from the local market) as the sole lipid source. These three groups received drinking water with 30% sucrose. Diets were prepared using BHT at 0.02% as antioxidant, and stored under refrigeration until the end of the study. Animals were checked for body weight and blood pressure after the 6-week feeding period.

Before sacrifice, blood samples were obtained from nonfasted animal's tails for nonfasting triglyceride measurement. Then, fasted animals were killed by decapitation

Table 1
Composition of diets administered to rats

Ingredient	Corn-canola oil diet (g)	Fish oil diet (g)	Corn oil diet (g)
Casein	440	440	440
DL-methionine	3.2	3.2	3.2
Cellulose	40	40	40
Starch	658	658	658
Vitamin mix[a]	20	20	20
Mineral mix[b]	80	80	80
Corn oil	50	–	100
Canola oil	50	–	
Fish oil		100	–

[a] 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.
[b] Mineral mix (Teklad AIN-76 170915): $CaHPO_4$, NaCl, potassium citrate, K_2SO_4, MgO, manganous carbonate, ferric citrate, zinc carbonate, cupric carbonate, potassium iodate, sodium selenite, chromium potassium sulfate, sucrose.

Table 2
Fatty acid composition of diets administered to rats

Fatty acid	Chow diet (%)	Corn-canola oil diet (%)	Fish oil diet (%)	Corn oil diet (%)
Saturated				
10:0	nd	0.05	0.07	0.02
12:0	0.2	nd	nd	nd
14:0	2.2	nd	14.4	nd
16:0	26.6	12.8	21.9	15.6
18:0	7.3	nd	2.3	nd
20:0	0.05	nd	6.5	5.5
Total	36.3	12.8	45.1	21.1
Monounsaturated				
14:1	0.44	nd	nd	nd
16:1	nd	nd	9.1	nd
18:1	50	50.7	13.9	31.8
20:1	2.7	nd	nd	6.6
24:1	nd	nd	2.6	nd
Total	53.1	50.7	25.6	38.4
Polyunsaturated				
n-6				
18:2	8.3	32.8	1.4	40.2
20:4	nd	nd	1.1	nd
Total	8.3	32.8	2.5	40.2
n-3				
18:3	1.7	3.5	0.96	nd
18:4	nd	nd	2.05	nd
20:5	nd	nd	11.2	nd
22:6	nd	nd	11.9	nd
Total	1.7	3.5	26.1	nd
n-6/n-3	4.8	9.3	0.09	nd

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

(without anesthesia to avoid interference with insulin measurements). Blood samples were taken to measure serum glucose, insulin, triglyceride, cholesterol, total lipids, HDL cholesterol and LDL cholesterol.

2.4. Blood pressure measurement

Systolic blood pressure was estimated by a tail-cuff method (IITC Noninvasive blood pressure system, Mod 29, Inc./Life Science Instruments, CA) in conscious animals. The reported blood pressure value is the mean of five systolic measurements.

2.5. Biochemical measurements

Total cholesterol was measured using enzymatic reagents [15]. Serum HDL was determined after precipitation of LDL and very low density lipoprotein (VLDL) cholesterol with phosphotungstic acid in the presence of Mg+ ions [16]. In a similar way, LDL cholesterol was determined after heparin precipitation of HDL and VLDL cholesterol [17]. A peroxide-coupled method for the colorimetric determination of serum triglyceride was used [18]. Serum glucose determination was done by using a color reagent under an oxidase system [19]. Total lipids (color test method), were performed according to the 3321 Merck test. Insulin levels were determined by a solid-phase ^{125}I radioimmunoassay (Coat-A-Count, DPC). The intra-assay variance for determining cholesterol, HDL, LDL, triglyceride, glucose and insulin were 2.2%, 2.4%, 1.6%, 1.9%, 2.1% and 2%, respectively.

2.6. Lipids isolation

Lipids were extracted from diets according to Folch et al. [20]. Fatty acids were converted to methyl esters by BF_3-catalyzed transmethylation [21], and analyzed by gas chromatography using a Hewlett Packard model 5890 Chromatograph equipped with a Carbowax capillary column and a flame ionization detector. Injection and detector temperatures were 250°C, helium was the carrier gas and the column temperature was programmed to rise from 130°C to 240°C at a rate of 8°C/min. Fatty acid methyl esters were identified by comparison with fatty acid standards (Sigma Chemical Co.).

2.7. Statistical analysis

Values are expressed as means ± S.E.M. Statistical significance was tested by analysis of variance procedures. A Tukey's multiple range test was used for mean comparison. Differences having *P*-values lower than .05 were considered to be statistically significant.

Table 3
Blood pressure, body weight and serum parameters in rats fed the chow (C) and chow plus high sucrose (C+HS) diets during 13 weeks

Parameters	C[a]	C+HS[b]
Systolic blood pressure (mmHg)	120.2 ± 2.5	139.0 ± 1.7**
Body weight (g)	337.2 ± 5.0	314.3 ± 1.3
Glucose (mg/dl)	139.1 ± 6.2	135.0 ± 8.8
Insulin (μUI/ml)	6.6 ± 1.6	14.8 ± 1.5**
Nonfast triglyceride (mg/dl)	94.5 ± 18.3	175.7 ± 15.4*
Fast triglyceride (mg/dl)	81.7 ± 7.5	110.7 ± 9.4
Cholesterol (mg/dl)	49.1 ± 0.4	61.2 ± 0.6**

[a] Values are means ± S.E.M. ($n = 18$).
[b] Values are means ± S.E.M. ($n = 72$).
* $P < .05$; ** $P < .01$.

3. Results

3.1. Experimental model

The model was achieved by the administration of 30% sucrose in drinking water in male Wistar rats during 13 weeks. Table 3 shows the values of blood pressure, body weight and serum concentrations of glucose, insulin, triglyceride and cholesterol in C-fed and C+HS-fed rats. Results showed that C + HS-fed rats increased systolic blood pressure levels (16%, $P < .01$) when compared with C-fed rats. Serum insulin, nonfasting serum triglyceride and serum cholesterol were also significantly increased (124%, 86% and 25%, respectively). These animals had a slight increase in fasting serum triglyceride, although not statistically significant. If fasting insulin level is used as an index of insulin resistance, as suggested recently for normoglycemic populations [22], our rat model exhibited insulin resistance. C + HS-fed rats and the C-fed rats presented similar body weight and serum levels of glucose. These results were according to our expectations and similar to those found in the literature that reported similar increases in the parameters evaluated after sucrose or fructose feeding [10–12].

3.2. Experimental diets

When the CVS-induced rat model was established, the effects of different dietary fat diets were analyzed. Table 4 reports the results observed in animals after administration of CC, FO and CO diets during 6 weeks.

Blood pressure in animals receiving the CC + HS diet was significantly higher (16%) than that of animals under the CC diet. The FO + HS group presented blood pressure levels similar to those found in the CC group, being only 3% higher than the CC group and significantly lower than the CC + HS group. The CO + HS group presented significantly higher levels (13%) of blood pressure than the CC group but similar to those found in the CC+HS group.

Similar behavior was found for serum insulin levels. Animals under the CC + HS diet showed a significant increase in insulin levels (63%) with respect to those in CC

Table 4
Blood pressure and serum insulin and triglyceride levels in rats fed the different dietary fat diets during 6 weeks

Parameters	CC	CC+HS	FO+HS	CO+HS
Systolic blood pressure (mmHg)	109.3 ± 3.4	127.3 ± 2*	113.4 ± 2**	123.4 ± 3.5*
Insulin (μUI/ml)	7.8 ± 0.6	12.7 ± 0.9*	8.7 ± 0.6**	11.8 ± 1.3*
Fasting triglyceride (mg/dl)	70.8 ± 3.5	150.6 ± 15.3*	90.6 ± 7.6**	94.6 ± 9.6**

Values are means ± S.E.M. (n = 18). CC, corn-canola diet; CC + HS, corn-canola diet plus 30% sucrose in drinking water; FO + HS, fish oil diet plus 30% sucrose in drinking water; CO + HS, corn oil plus 30% sucrose in drinking water.
* $P < .01$ with respect to CC; ** $P < .01$ with respect to CC + HS.

rats. Insulin levels in the FO + HS animals were 12% higher than those of the CC group, but significantly lower than the CC + HS group. The increase in insulin levels in the CO + HS animals with respect to the CC group resembled that found in the CC+HS group (51%). A significant triglyceride increase was found in the CC + HS group with respect to the triglyceride level in the CC group (112% increase). FO-fed and CO-fed rats showed significantly lower levels of triglycerides than those fed the CC + HS diet, which were statistically not different to the CC group.

Glucose levels were elevated in all experimental groups (about 20%), although not significantly different from the CC group. Serum levels of HDL, LDL, total lipids and cholesterol as well as body weight were not significantly modified by the diets (data not shown).

4. Discussion

By means of sucrose administration in drinking water (30%), a group of rats with metabolic abnormalities (hypertriglyceridemia, hyperinsulinemia and mild hypertension) similar to those found in the so-called CVS was developed. Our results are similar to those reported previously in which the administration of fructose- or sucrose-rich diets to experimental animals caused comparable degrees of hyperinsulinemia, hypertriglyceridemia, insulin resistance and blood pressure elevation [10–12].

The clustering of these metabolic abnormalities allowed us to use this group of rats as an integrative experimental model to analyze the effects of the high-PUFA diets on these parameters of CVS.

The FO diet, high in n-3 PUFA, provoked a significant reduction in the three parameters that were found significantly elevated after sucrose feeding, returning them to levels similar to those found in control rats.

FO and, in particular, long chain n-3 PUFA, have been shown to reduce blood pressure in normotensive [23–25] and hypertensive subjects and in experimental animals [26–35], although some studies have failed to demonstrate any such effect [36–40]. The detailed mechanisms mediating this antihypertensive effect are unknown, but they have been attributed to a shift in eicosanoid production away from the 2-series prostaglandins derived from arachidonic acid that are potent vasoconstrictors as well as dilators to the 3-series prostaglandins derived from eicosapentaenoic acid (the 20-carbon n-3 equivalent of arachidonic acid) that are either inactive or vasodilators [6,41,42]. However, there are a number of epidemiological, physiological and biochemical studies that demonstrate a strong positive relationship between elevated insulin levels (which implies underlying insulin resistance) and hypertension [43–45]. There are a number of potential mechanisms by which insulin can induce hypertension (sympathetic nervous system activation, renal sodium retention, altered cellular electrolyte composition and stimulation of cell growth) [44]. Evidence that links insulin resistance and hyperinsulinemia to the development of hypertension shows that manipulations aimed to create a state of insulin resistance in experimental animals lead 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 levels also decrease blood pressure [44,45]. In our study, FO feeding caused a significant reduction in serum insulin levels in rats, so this reduction could be responsible of the lowering effect found on blood pressure.

Additionally, the alterations in the fatty acid composition of the tissue membranes after diet administration (unpublished data) could alter membrane-mediated processes such as insulin transduction signals, activity of lipases and synthesis of eicosanoids and, in this way, affect insulin sensitivity, blood pressure and triglyceride synthesis [22,46–48].

With respect to n-6 PUFA actions on blood pressure and hyperlipidemias, the literature evidence is not conclusive. In some instances, linoleic acid has been associated with increases or no effect on blood pressure levels [6,49–52], but a hypotensive effect has also been reported with high intakes of n-6 fatty acids [53–56]. It has been suggested that the effect depends on the model utilized and on the particular fatty acid being administered [49,50,57].

In our study, the CO diet (rich in linoleic acid) provoked a slight, although not statistically significant, reduction in blood pressure levels with respect to control animals. Insulin levels were similar to those found in the sucrose-fed group, in accordance with reports of insulin resistance induced by a high-fat diet (rich in n-6 PUFA) and the worsening of insulin action by increasing dietary n-6/n-3 ratio [22].

The effects of dietary n-6 PUFA on triglyceride levels

are poorly investigated, and there is inconsistency in the results [26,58]. In our work, rats receiving the CO diet had a 40% reduction in triglyceride levels.

It is interesting to notice that Western diets are characterized by high intakes of n-6 PUFA due to dietary habits that include high intakes of edible oils (corn, soybean, sunflower, canola). Because the same desaturase-elongase system works in both PUFA family precursors (18:2 and 18:3), the metabolites from 18:2 are the most prevalent, especially when 18:3 n-3 intakes are very low. This imbalance in essential fatty acids intakes (extremely high n-6 and very low n-3) in the Western population could well be correlated to the high incidence of non-insulin-dependent diabetes mellitus and cardiovascular syndrome that prevails in these countries.

In conclusion, we obtained an animal model for hypertension, hyperinsulinemia and hypertriglyceridemia by means of sucrose administration in drinking water (30%). The effects of sucrose on blood pressure were reverted by the FO diet. The effects on triglyceride levels were reverted by both dietary oils (FO and CO), and the effects on insulin levels by FO. We suggest that dietary n-3 PUFA supply may have favorably influenced the parameters analyzed by improving insulin action, which is another possible mechanism for explaining the beneficial effects of FO on cardiovascular diseases by means of the modulation of the damaging cardiovascular effects of insulin.

The results obtained in this work highlight the importance of conducting large epidemiological studies aimed to test the efficacy of dietary fat manipulation on CVD.

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