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Effect of sucrose addition to drinking water, that induces hypertension in the rats, on liver microsomal Δ9 and Δ5-desaturase activities

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Abstract

This study was undertaken with the aim of investigating the effect of sucrose addition to the drinking water of rats who were fed with the same diet as a control group, on Δ9- and Δ5-desaturase activities and on the fatty acid composition of serum and liver microsomes. Weanling male Wistar rats had 30% sucrose in their drinking water for 20 weeks. An increase in total calories consumed, visceral fat accumulation, insulin, triglycerides and blood pressure and a decrease in the food intake were observed in the sucrose-fed group as compared with the control group. A decrease in linoleic and α-linolenic acid (essential fatty acids) in all serum lipid fractions of sucrose-fed rats was found. This observation correlated with a low food intake by sucrose-fed rats. The conversion of [1 ^{14}C]-palmitic to [1 ^{14}C]-palmitoleic acid by Δ9-desaturase activity was increased in sucrose-fed compared with control rats, while the conversion of [1 ^{14}C]-dihomo-γ-linolenic acids by Δ5-desaturase activity was depressed. In sucrose-fed as compared to control rats, the proportion of palmitoleic and oleic fatty acids was increased. Arachidonic acid was decreased in sucrose-fed rats. The 1,6-diphenylhexatriene fluorescence polarization of the microsomal membranes was significantly lower in the sucrose-fed group compared to the control group. These results indicate that the sucrose addition to the drinking water of the rats increased microsomal Δ9-desaturase activity and membrane disorder and decreased the activity of the Δ5-desaturase, a key enzyme in the biosynthesis of arachidonic acid, implicated in hypertension.

Keywords: Sucrose intake; Desaturase activities; Hypertriglyceridemia; Hypertension

1. Introduction

The intake of a diet rich in fructose or sucrose has long been known to result in elevated serum triglycerides in both humans and experimental animals [1–3]. So far, most animal studies on the mechanism underlying carbohydrate-induced hypertriglyceridemia and alterations in the fatty acid (FA) metabolism, have been carried out using a diet rich in carbohydrate with the same proportion of the nutrients as the control diet, except that the carbohydrates were replaced with starch [4,5]. These studies described that the administration of carbohydrates and the observation of its effect on the fatty acid metabolism in animals was carried out for periods of short- or medium length (a few weeks) of carbohydrate ingestion, which are not sufficient to cause high blood pressure in the animals. Studies on the long-term metabolic consequences of sucrose addition to the water that induces hypertension in animals, are limited particularly to those concerning fatty acid modifications of serum and membrane phospholipids of microsomes from rat liver. Polyunsaturated fatty acids (PUFA) are major constituents of the membrane phospholipid bilayer, since PUFA composition is the most important factor that influences the membrane physical properties and related cell functions [6,7]. Changes in the fatty acid composition of several tissues can be attributed to the type of fat ingested in the diet or to the alterations in the metabolism such as their oxidation, their incorporation in the membrane phospholipids or to the desaturase activities, enzymes implicated in the biosynthesis of the PUFA. Considering the important role of n-6 PUFA and their oxygenated metabolites in hypertension, it has been shown that normal and hypertensive animals on an essential fatty acid-deficient diet, such as that of a linoleic and arachidonic acid-deficient diet, are prone to blood pressure elevation [8,9]. A modification in arachidonic acid proportion in tissues could suggest an alteration

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Table 1
Fatty acid composition of the diet

Fatty acids	Composition (% of total identifiable fatty acids)
C16:0	26.9
C16:1n-7	3.0
C18:0	19.1
C18:1n-9	27.3
C18:2n-6	22.4
C18:3n-6	0.3
C18:3n-3	0.4
Other	0.6
SFA	46
MUFA	30.3
PUFA(n-6)	22.7

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

in the bioconversion of linoleic acid to arachidonic acid. A direct study of the enzymatic bioconversion of this fatty acid to the long PUFA permits us to investigate the mechanisms by which sucrose added to the water induces the alterations in the fatty acid metabolism observed in rats [10,11]. The desaturation of the fatty acids is an important factor in the regulation of the membrane disorder, a state that strongly influences the behavior of integral proteins. The physical state of the membrane can be investigated by the fluorescence polarization of 1,6-diphenylhexatriene (DPH), a method that has been frequently used to study the rate and the range of fatty acids wobbling in the membrane [12]. In the present work we studied the effect of sucrose added to the water of rats on *in vitro* desaturase activities and alterations in the fatty acid composition that produces changes in the physical state of their liver microsomal membrane.

2. Methods and materials

2.1. Animals and diet

Weanling male Wistar rats aged 28 days and weighing approximately 45 ± 2 g were housed with a 12-h light-dark cycle and randomly separated into two groups of 6 animals: Group I (Control), rats given tap water for drinking and Group II (sucrose-fed) rats given 30% sucrose in their drinking water for periods of approximately 20 weeks. All animals were fed with commercial rat chow (PMI Nutrition International, Inc., LabDiet 5008, Richmond, IN, USA) ad-libitum. Chow-diet contained: 23.5% of crude protein; 3.8% of crude fiber; 6.8% of ash; 2.5% of mineral; 6.5% fat from animals and vitamins: 4 ppm carotene, 15 U/g vitamin A, 3.3 U/g vitamin D, 55 U/g vitamin E. Fatty acid composition of the diet is shown in Table 1. The end of the sucrose treatment period was taken as the time when the blood pressure was significantly increased in at least 80% of the sucrose fed animals. All animals had free access to food and water. Animals were studied in compliance with our institution's guidelines for animal research. Monthly measurements of the rats' arterial pressure were taken with the tail-cuff method as described previously [13]. Visceral fat was dissected as described by Belzung et al. [14].

2.2. Blood samples

At the end of the treatment period, the animals were fasted overnight and then killed by decapitation. Blood samples were collected taking care to avoid haemolysis. The blood was centrifuged at 1086 × g for 10 min and serum was separated. The triglycerides were measured by means of an enzymatic technique (Boehringer Mannheim, Mannheim, German) in an Abbott VP Series II Autoanalyzer (Irving, TX), according to the method described by Nägele et al. [15]. Insulin was measured by immunoassay (Boehringer Mannheim).

2.3. Isolation of microsomes

Liver microsomes were prepared as reported in the literature [16]. The liver was removed and placed in ice-cold homogenizing solution containing: 0.25 mol/L sucrose, 0.1 mmol/L EDTA, 62 mmol/L potassium phosphate buffer, 0.15 mol/L KCl, 5 mmol/L $MgCl_2$ and 1 mmol/L DTT (final pH 7.4). The liver was cut into thin slices and homogenized using a glass teflon-homogenizer. Cell debris and nuclei were removed by centrifugation at 754 × g for 10 min, then the supernatant was centrifuged at 12 062 × g for 10 min to eliminate the mitochondrial fraction. The resulting supernatant was used for the isolation of the microsomal fraction by centrifugation at 100 000 × g during 45 min. All these operations were carried out at 4°C. The pellet was suspended in the homogenizing buffer at 20 mg protein by ml and stored at −70°C. The purity of the microsomal fraction was assessed by measuring glucose-6-phosphatase and succinate dehydrogenase for their contamination by mitochondrial fraction [17]. Protein was measured by the method of Lowry et al. [18], using bovine serum albumin as standard.

2.4. Lipid extraction and fatty acid composition profile

Five hundred μl of serum, containing 226 μmol/L BHT as antioxidant and stored at −70°C were used for the extraction of lipids as described by Folch et al. [19] in the presence of phosphatidylcholine-diheptadecanoyl, 1,2,3-triheptadecanoylglycerol and cholesterol-3-heptadecanoate as internal standards for phospholipids (PL), triglycerides (TG) and cholesterol esters (CE), respectively. All solvents used for the extraction contained 0.005% BHT as an antioxidant. The lipid extracts were separated by monodimensional thin layer chromatography (TLC) on silica gel 60G using a mixture of hexane-ether-formic acid (80:20:2, by vol.). Lipid standards were run in parallel to identify the

various lipid classes and were visualized by exposure to iodine vapors. The lipid fractions such as PL, TG and CE, not exposed to iodine, were scraped off and were transesterified to their FA methyl esters as described by Christie [20]. The lipid fractions were transesterified to their fatty acid methyl esters by heating at 80°C for 2 hours with MeOH, containing 2% concentrated H_2SO_4 and 0.005% BHT. Fatty acid methyl esters were separated and identified by gas liquid chromatography in a Carlo Erba model 2300 chromatograph, fitted with a 25 m × 0.25 mm i.d. fused-silica capillary column, which was coated with CP-Sil 88 (film thickness 0.25 μm). The analysis was carried out at 195°C (oven temperature), using helium gas as a carrier, at a flow rate of 1 ml/min.

The microsome fraction, containing BHT as antioxidant (226 nmol/10 mg protein), was stored at −70°C until the lipid analysis was carried out. Ten mg of microsome protein were used for the extraction of lipids in the presence of phosphatidylcholine-diheptadecanoyl, as an internal standard [19]. The phospholipids were separated from neutral lipids by thin layer chromatography on silica gel 60G. The phospholipid fractions, not exposed to iodine, were scraped off and transesterified to their fatty acid methyl esters as described above. Phospholipid quantification was carried out as reported in the literature [21].

2.5. Enzymatic assays

The conversion of [1 ^{14}C]-dihomo-γ-linolenic and of [1 ^{14}C]-palmitic acid to their corresponding products was performed as described in the literature [22]. The desaturase assay was carried out at 30°C for 10 min, in a final volume of 1.5 ml of a solution containing: 0.04 mmol/L potassium phosphate buffer (pH 7.0), 0.70 mmol/L gluthatione, 1.3 mmol/L ATP, 0.06 mmol/L coenzyme A, 0.87 mmol/L NADH, 0.04 mmol/L NaF, 0.33 mmol/L nicotinamide, 5 mmol/L $MgCl_2$, 200 nmol dihomo-γ-linolenic acid (1000 d.p.m./nmol) and palmitic acid (1080 d.p.m./nmol) in the presence of 5 and 3 mg protein, respectively. At the end of the incubation time, the reaction was stopped by addition of 2 mL 10% methanolic KOH and mixed vigorously. The resulting mixture was saponified at 70°C for one hour. Afterwards the medium was acidified with 2 mol/L HCl. Fatty acids were extracted with petroleum ether and esterified to their corresponding methyl esters. The products of the desaturation were separated from their corresponding reagents by TLC on silicagel 60G impregnated with 10% silver nitrate. In the desaturation experiments, fatty acid methyl esters obtained from total microsome lipids, were fractionated by argentation TLC using a solvent system, hexane-diethyl ether (85:15, by vol), to separate dienes and monoenes from saturated fatty acids. Diethyl ether-methanol (90:10, by vol) was used as a solvent system to separate tetraenes from trienes [23]. The different fatty acid fractions were visualized with 2′, 7′-dichlorofluorescein and scraped off, the radioactivity was determined by liquid scintillation counting.

2.6. Determination of fluorescence polarization

The fluorescence polarization was analysed, at 30°C, in microsomes (2 mg protein) incubated in 2 ml of media containing 0.25 mol/L sucrose adjusted to pH 7.3 with Tris base. In addition, the media contained 1 mmol/L DPH solubilized in dimethylformamide. The mixture was incubated at 25°C during 30 min. Each sample was subjected to polarization analysis in an Aminco Bowman fluorometer (340 nm excitation—417 nm emission) [12].

2.7. Data analysis

Statistical analysis was performed by means of a statistical and graphic system (SigmaPlot, version 2.01, Jandel Corporation, 1986–1994). Data are presented as the mean ± SD. Statistical significance was determined by the Student's t test ($p < 0.05$).

2.8. Materials

[1 ^{14}C]-dihomo-γ-linolenic (54 mCi/mmol) and [1 ^{14}C]-palmitic acid (45 mCi/mmol) were purchased from Amersham International (Amersham, England). Unlabeled fatty acid, butylated hydroxy toluene (BHT), coenzyme A, bovine serum albumin, free fatty acids, ATP, and NADH were purchased from Sigma Chemicals Co. (St Louis, MO, USA). All the other chemicals were of analytical grade.

3. Results

Table 1 shows the fatty acid composition of the diet. It contained a relatively large amount of linoleic acid (22.4%), an essential fatty acid and a precursor of arachidonic acid

Table 2
Characteristics of the rat groups

Variables	Control	Sucrose-fed
Liquid consumption (mL/d)	40 ± 6	33.7 ± 3.6
(equivalent in Kcal)	0	40.4 ± 4
Food consumption (g/d)	17.5 ± 1	13.9 ± 0.8***
(equivalent in Kcal)	61.2 ± 6.5	48.6 ± 2.8***
Total Kcal	61.2 ± 6.5	89.0 ± 0.8***
Body weight (g)	433.00 ± 42.27	410.90 ± 31.81
Visceral fat (g)	13.2 ± 1.59	34.6 ± 7.59***
Triglycerides (mmol/L)	0.79 ± 0.34	1.73 ± 0.89*
Blood Pressure (mm Hg)	113.76 ± 13.64	148.59 ± 10.45**
Insulin (mU/mL)	7.6 ± 0.6	11.5 ± 1.9*

Values are means ± SD ($n = 6$).
*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$.

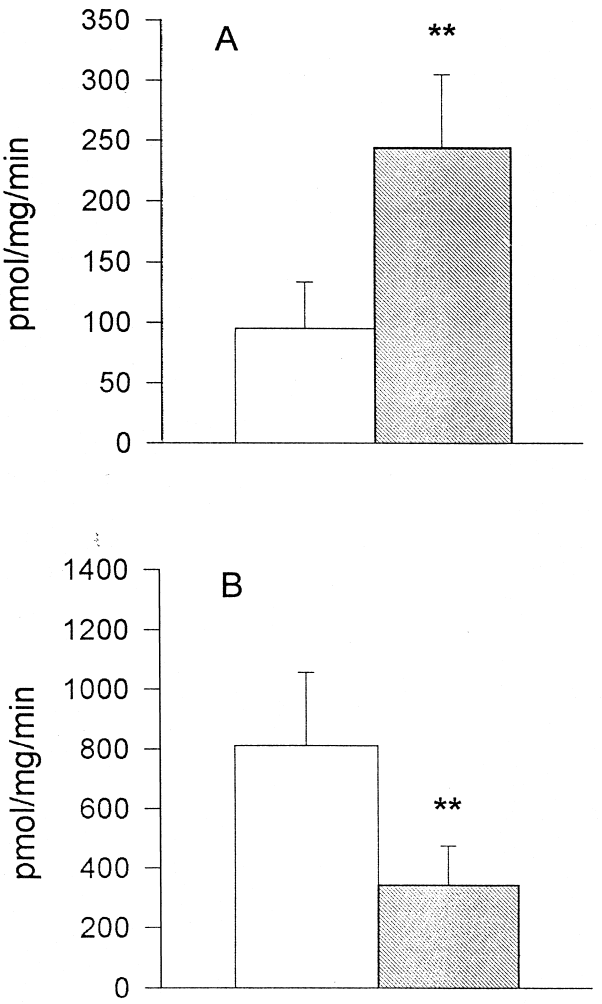

Fig. 1. Influence of dietary sucrose on Δ9 (1A) and on Δ5-desaturase activities (1B) in rat liver microsomes. Shaded bars correspond to the sucrose-fed group and open bars correspond to the control group. Enzyme activities were expressed as pmol of labeled palmitoleic and arachidonic acid converted from palmitic and dihomo-γ-linolenic acids per minute per mg of microsomal protein. The results are means ± SD of six separate microsomal preparations from six different rats. *$P < 0.05$; **$P < 0.01$.

biosynthesis. Both groups of animals ingested the same type of fatty acids.

Table 2 shows no difference in the liquid consumption between the two groups but a significant decrease in food and caloric intake in sucrose-fed rats. Under these conditions, we found that the ingestion of a high amount of sucrose led to a significant increase in the level of TG ($p < 0.05$) and insulin ($p < 0.05$) in the serum and a significant increase of blood pressure (BP) ($p < 0.01$) and in visceral fat accumulation ($p < 0.001$).

Fig. 1 shows the desaturation enzyme activities. The conversion of palmitic to palmitoleic acid by Δ9-desaturase activity (Fig. 1A) was increased by 155% in sucrose-fed rats compared with the control, while the conversions of dihomo-γ-linolenic acids by Δ5-desaturase (Fig. 1B) activity, were depressed in sucrose-fed rats by 57%. Those alterations of Δ9 and Δ5-desaturation were reflected in the fatty acid composition of serum and liver microsomes.

Table 3
Fatty acid composition of phospholipids of liver microsomes in sucrose-fed and control rats

Fatty acids	Control group	Sucrose-fed group
C16:0	23.44 ± 4.09	31.64 ± 2.54**
C16:1n-7	1.73 ± 0.27	3.76 ± 0.84**
C18:0	23.79 ± 4.31	20.81 ± 3.53
C18:1n-9	8.55 ± 1.86	16.81 ± 3.80**
C18:2n-6	21.35 ± 3.75	10.44 ± 2.98**
C18:3n-6	0.10 ± 0.09	0.10 ± 0.11
C18:3n-3	0.13 ± 0.05	0.11 ± 0.07
C20:3n-6	1.20 ± 0.18	0.90 ± 0.13
C20:4n-6	18.56 ± 1.88	14.12 ± 2.65***
C20:5n-3	1.68 ± 0.81	0.43 ± 0.17**
C22:6n-3	1.03 ± 0.53	1.20 ± 0.54
SFA	47.24 ± 6.11	52.45 ± 4.77
MUFA	10.28 ± 2.07	20.58 ± 4.55**
PUFA	44.74 ± 5.45	28.26 ± 1.17***
C16:1n-7/C16:0	0.07 ± 0.01	0.12 ± 0.03**
C18:1n-9/C18:0	0.35 ± 0.03	0.84 ± 0.28*
C18:3n-6/C18:2n-6	0.005 ± 0.01	0.01 ± 0.03
C20:4n-6/C20:3n-6	15.86 ± 3.91	16 ± 4.55
C22:6n-3/C20:5n-3	0.69 ± 0.58	2.96 ± 2.65

Data represent the weight of each individual FA/weight of total FA as percentage (mean of % wt ± SD, $n = 6$). SFA, Saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
*** Significantly different from control group ($P < 0.001$)
** $P < 0.01$.
* $P < 0.05$.

In microsomes from sucrose-fed as compared to those from control rats, the proportions of palmitoleic and oleic fatty acids were increased by 34% and 96% respectively, while arachidonic acid was found decreased by 23% (Table 3). Linoleic acid was found in a lower proportion in sucrose-fed rats. The latter variations resulted in a significant increase of total monounsaturated fatty acids (MUFA) and in a significant decrease of total PUFA. In the case of saturated fatty acids (SFA), palmitic acid increased by 32% in sucrose-fed rats, while stearic acid was present at the same level in both groups. This result was not reflected in total SFA which were moderately increased in the sucrose-fed group without a statistically significance. As shown in Table 3, ratios C16:1n-7/C16:0 and C18:1n-9/C18.0 were significantly higher ($p < 0.01$ and $p < 0.05$ respectively) in sucrose-fed rats. No difference was observed in ratios C18:3n-6/C18:2n-6 and C20:4n-6/C20:3n-6 between sucrose-fed and control rats.

In the serum, lipid fractions (PL, TG and CE) were separated by TLC. Fatty acid composition of each is shown in Table 4.

In the PL fraction, sucrose feeding caused a significant increase in palmitoleic ($p < 0.01$), oleic ($p < 0.01$), and dihomo-γ-linolenic acid ($p < 0.01$) proportions and a significant decrease in linoleic ($p < 0.01$) and arachidonic acid ($p < 0.01$) proportions. An increase in palmitic acid

Table 4
Fatty acid composition of lipid serum fractions and some ratios of products/substrates in the sucrose-fed and control rats

	Phospholipids		Triglycerides		Cholesterol esters	
Fatty acids	Control	Sucrose-fed	Control	Sucrose-fed	Control	Sucrose-fed
C16:0	29.64 ± 3.48	36.37 ± 3.69*	31.98 ± 3.42	38.72 ± 4.34*	17.27 ± 2.15	24.35 ± 4.56**
C16:1n-7	0.84 ± 0.34	2.46 ± 0.81**	2.56 ± 1.08	5.48 ± 1.64**	2.96 ± 1.29	7.47 ± 2.41**
C18:0	22.14 ± 4.90	18.59 ± 3.46	7.95 ± 0.98	5.74 ± 1.73	4.98 ± 1.88	7.37 ± 5.44
C18:1n-9	6.37 ± 1.63	11.43 ± 2.64**	36.62 ± 1.63	42.52 ± 4.15**	9.52 ± 1.18	15.44 ± 4.29**
C18:2n-6	26.72 ± 3.00	18.31 ± 2.25**	18.72 ± 2.82	6.49 ± 2.04***	28.25 ± 5.24	16.21 ± 6.15**
C18:3n-6	0.20 ± 0.13	0.30 ± 0.21	0.22 ± 0.17	0.19 ± 0.10	0.67 ± 0.23	0.98 ± 0.09*
C18:3n-3	0.29 ± 0.22	0.15 ± 0.13	0.63 ± 0.19	0.26 ± 0.07**	0.62 ± 0.26	0.17 ± 0.39*
C20:3n-6	0.76 ± 0.30	1.63 ± 0.49**	0.15 ± 0.08	0.24 ± 0.12	0.44 ± 0.41	0.90 ± 0.57
C20:4n-6	10.49 ± 1.02	7.85 ± 1.11**	0.76 ± 0.30	0.33 ± 0.15*	32.21 ± 6.72	22.81 ± 6.27*
C20:5n-3	0.82 ± 0.22	0.74 ± 0.33	0.75 ± 0.48	0.51 ± 0.21	2.82 ± 1.76	2.24 ± 1.66
C22:6n-3	1.74 ± 0.52	2.59 ± 1.29	nd	nd	0.81 ± 0.22	1.16 ± 0.29*
SFA	51.78 ± 7.93	54.96 ± 2.97	39.93 ± 3.95	44.20 ± 4.95	22.76 ± 3.19	31.83 ± 9.72*
MUFA	7.21 ± 1.98	13.89 ± 1.80***	39.18 ± 2.71	47.99 ± 4.42*	12.48 ± 2.99	22.91 ± 4.51***
PUFA (n-6)	38.07 ± 8.74	28.01 ± 2.85*	20.05 ± 5.30	7.24 ± 2.22***	61.59 ± 5.97	41.01 ± 11.61**
PUFA (n-3)	2.85 ± 0.52	3.48 ± 1.33	1.17 ± 0.52	0.75 ± 0.29	5.19 ± 1.64	4.07 ± 1.46
C16:1n-7/C16:0	0.03 ± 0.01	0.07 ± 0.03**	0.08 ± 0.04	0.14 ± 0.04*	0.17 ± 0.07	0.32 ± 0.13*
C18:1n-9/C18:0	0.29 ± 0.08	0.65 ± 0.25**	4.67 ± 0.68	8.02 ± 2.82*	2.10 ± 0.07	2.89 ± 1.82
C18:3n-6/C18:2n-6	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01*	0.02 ± 0.01	0.06 ± 0.02**
C20:4n-6/C20:3n-6	13.80 ± 4.15	5.19 ± 2.37**	5.15 ± 2.04	1.39 ± 1.14*	110.53 ± 67.17	35.46 ± 27.13*

Data represent the weight of each individual FA/weight of total FA as percentage (mean of % wt ± SD) ($n = 6$ different animals).
*** Significantly different from control group ($P < 0.001$).
** ($P < 0.01$).
* ($P < 0.05$).
nd: not detected.

level was noted while the proportion of stearic acid was not significantly changed. These variations resulted in a significant increase of total MUFA and in a significant decrease of total PUFA. As shown in Table 4, ratios C16:1n-7/C16:0 and C18:1n-9/C18:0 were significantly higher ($p < 0.01$) in sucrose-fed rats while C18:3n-6/C18:2n-6 was not significantly different from that of control rats. In contrast ratio C20:4n-6/C20:3n-6 was significantly lower ($p < 0.01$) in sucrose-fed rats.

In the TG fraction we observed a significant increase in the palmitic ($p < 0.05$), palmitoleic ($p < 0.05$) and oleic ($p < 0.05$) proportions and a significant decrease in the linoleic, α-linolenic and arachidonic acids. These results indicate an increase in total MUFA ($p < 0.05$) and a decrease in the PUFA ($p < 0.001$) in sucrose-fed group. Ratios C16:1n-7/C16:0 and C18:1n-9/C18:0 were significantly higher ($p < 0.05$) in the sucrose-fed rats. In contrast, ratio C20:4n-6/C20:3n-6 was significantly lower ($p < 0.05$) in the sucrose-fed group.

In comparison with controls, the following changes were observed in the serum CE fraction of the sucrose-fed rats: A higher proportion of palmitic acid was found ($p < 0.01$). As observed in PL and TG fractions, the proportions of palmitoleic and oleic acid were significantly higher ($p < 0.01$, and $p < 0.05$ respectively). In the case of PUFA such as linoleic, α-linolenic and arachidonic acids, they were present in a significantly lower proportion. A significant increase ($p < 0.05$) in the proportion of docosahexaenoic acid was observed in sucrose-fed as compared with the control rats. These results indicate an increase of total SFA ($p < 0.05$) and MUFA ($p < 0.001$) and a decrease in the PUFA ($p < 0.01$). Ratio C16:1n-7/C16:0 was significantly higher while no significant difference was observed in the ratio C18:1n-9/C18:0 between both groups. Ratio C18:3n-6/C18:2n-6 was significantly higher whereas C20:4n-6/C20:3n-6 was significantly lower, in sucrose-fed rats as compared with control rats.

The effect of sucrose feeding on the fluorescence polarization of DPH and on the relative percentage of cholesterol and phospholipids, factors which can influence the physical state of the microsomal membrane, is shown in Table 5. There are no statistical differences in cholesterol and phos-

Table 5
Fluorescence polarization of DPH and lipid profile in liver microsomes from sucrose-fed and control rats

Variables	Control	Sucrose-fed
Chol (μmol/mg prot.)	44.0 ± 2.8	48.4 ± 6.5
PL (μmol/mg prot.)	541 ± 25	585 ± 33
Chol/PL	0.77 ± 0.24	0.83 ± 0.01
SFA/MUFA	4.69 ± 0.79	2.73 ± 1.07**
SFA/PUFA	1.09 ± 0.24	1.77 ± 0.16***
Polarization	0.200 ± 0.002	0.190 ± 0.008*

Results are the mean ± SD of six animals.
Chol., Cholesterol; PL, Phospholipids.
*** Significantly different from control group ($P < 0.001$).
** $P < 0.01$.
* $P < 0.05$.

pholipid content. The cholesterol-phospholipid ratio remained constant under the dietary regime applied in our study. This ratio was calculated in view of the influence of this variable on the membrane disorder of the lipid bilayer [24]. However, an increase in the total MUFA and a decrease in the total PUFA observed in liver microsomes from sucrose-fed rats were reflected in ratios SFA/MUFA and SFA/PUFA that were significantly lower and higher in sucrose-fed group, respectively. The DPH fluorescence polarization of the microsomal membrane, measured at 30°C, was significantly lower in sucrose-fed compared with the control group. The lower polarization reflects the higher microsomal membrane disorder.

4. Discussion

The purpose of this study was to investigate the mechanism by which a long sucrose ingestion period induces alterations in the cell membrane fatty acid composition and the possible relationship between sucrose-induced hyperinsulinemia and hypertension and changes in cell membrane fatty acid composition, which could influence those biochemical processes involved in the development of hypertension.

Sucrose added to the drinking water of the rats induces a significant increase in TG, in insulin concentrations and in BP. There was no statistically significant difference in the weight between control and sucrose-fed rats, coinciding with what is described in the literature [25,26]. The lack of weight difference between the two groups might be explained by lower food intake by the sucrose-fed in comparison with the control animals. The body composition was not examined in detail, but a significant increase in visceral adipose tissue was noted in sucrose-fed rats due to the high energy intake (Table 2). This observation agrees with that described by Toida et al. [26] Visceral fat accumulation has been found to be closely related to hyperinsulinemia, hyperlipidemia and hypertension [27]. Nevertheless, direct implication of the increase in visceral fat, induced by sucrose feeding in the rats, in the development of hypertension has not been demonstrated and needs further investigation.

The analysis of the fatty acid composition showed a significant modification in the FA metabolism such as an accumulation of palmitic acid in serum; its β-oxidation may be depressed, while its biosynthesis and its esterification into PL, TG and CE may be concurrently elevated in the liver. High proportions of palmitoleic and oleic were found in all serum lipid fractions analyzed, indicating a probable increase of the Δ9-desaturase activity. The increase in blood pressure might be relative to the increase in oleic acid level. Oleic acid has been reported to exert a pressor effect in rats when administered by intravenous infusion [28].

One of the most frequently observed alterations in serum from humans with essential hypertension, and in experimental hypertensive animals, is the reduction of linoleic acid content as described in our present study [29,30]. Indeed, linoleic acid as 6-desaturated essential fatty acid has been found to lower blood pressure in hypertensive humans [31,32] and attenuate the development of hypertension in spontaneously hypertensive rats [33], when administered in the diet. In our sucrose-fed rats, the lower proportion of linoleic acid could be attributed to its deficient uptake from the diet. Alpha-linolenic acid also has been found in lower proportion in sucrose-fed rats as compared to the control group. Enhanced dietary intake of alpha-linolenic acid decreases blood pressure in spontaneously hypertensive rats and increases prostacyclin formation [34]. On the other hand, a deficiency in essential fatty acids and proteins induces alterations in the Δ9 and Δ5-desaturase activity in the rats [35,36]. Desaturases are key enzymes in the biosynthesis of the monounsaturated and PUFA and thereby contribute to the control of the fatty acid-dependent structure and disorder of the membrane. A relationship has been observed between the C16:0/C16:1n-7 and C18:0/C18:1n-9 ratios (usually used as indices of *in vivo* Δ9-desaturase activity) and *in vitro* Δ9-desaturase activity. The significant increase in the proportion of palmitoleic and oleic acids may reflect the *in vivo* higher activity of Δ9-desaturase. An increase in the Δ9-desaturase activity has been reported to be related to hyperinsulinemia [37,38]. Indeed insulin has been found to induce an over-expression of Δ9-desaturase in animals as well as in cell cultures [39,40]. A significant increase in the conversion of palmitic to palmitoleic acid by Δ9-desaturase *in vitro*, may be attributed to a high level of the enzyme in microsomes, due to the hyperinsulinemia observed in the sucrose-fed rats. Δ5-desaturase activity, key enzyme in the biosynthesis of the arachidonic acid, is depressed in the sucrose-fed rats. The decrease of the Δ5-desaturase activity *in vitro* was indirectly shown by the pattern of serum and liver microsomal fatty acid composition, with a decrease in the proportion of arachidonic acid. The proportion of dihomo-γ-linolenic acid, a direct precursor of arachidonic acid, was higher in serum phospholipids, which can be related to the decrease in the Δ5-desaturase activity and a lower proportion of arachidonic acid in the sucrose-fed rats. On the other hand, the reduction in arachidonic acid biosynthesis could be a determining factor that may account for an altered synthesis of arachidonic-derived prostaglandins (PGE_2, PGI_2, TXA_2), involved in the regulation of vascular tone. Indeed Δ5-desaturase activity has been found to be lower in spontaneously hypertensive rats [41]. Thus an imbalance in the distribution of the C20 PUFA (dihomo-γ-linolenic, arachidonic and eicosapentaenoic) can contribute to the development and the maintenance of hypertension in sucrose-fed rats.

Alterations in the fatty acid composition of the microsomal membrane affect its physical state. The effect of the number and position of double bonds situated along the fatty acyl chain of phospholipids, on the physical properties of the lipid bilayer, has been well studied [42]. A significant difference observed in the DPH fluorescence polarization

reflects a high membrane disorder of the liver microsome membrane in the sucrose-fed rats, that can alter the kinetic biosynthesis of PUFAs [43,44]. However, the change in the fatty acid composition observed in liver microsomes and characterised by an increase in palmitoleic and oleic acids, corresponds to the physical change observed in the lipid bilayer of microsomes, induced by sucrose feeding. The disorder of the membrane could be the result of a high proportion of monounsaturated fatty acids in the microsomal membrane as observed in microsomal membrane of liver from spontaneously hypertensive rats [45]. These fatty acids have been found to induce a high membrane disorder in liposomes constituted by phospholipids containing a specific fatty acid [42]. Other parameters, which can modify the physical state of the membrane and can induce changes in desaturase activities such as cholesterol and phospholipid ratios, were not changed in the sucrose-fed as compared with the control rats.

With the present data we are not able to define entirely the direct cause of blood pressure elevation induced by a high ingestion of sucrose in the rats. However, since membrane function is one of the most important physiological processes, our findings show far-reaching involvement of the alteration of membrane fatty acid composition and of desaturase activities in the elevated blood pressure. Nevertheless, other factors such as psychosocial stress [46] and physiological conditions such as hormonal balance, nutritional factors and aging [47,48] can modulate the activity of the desaturase enzyme and thereby contribute to the regulation of the biosynthesis of the immediate precursor of prostaglandin production implicated in the pathogenesis of hypertension.

Our results provide information on possible mechanisms implicated in the elevation of blood pressure, based on the alterations in the essential fatty acid metabolism due to high ingestion of sucrose added to the drinking water, which is associated with deficiency in food intake.

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