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Basic nutritional investigation

Changes in lipid metabolism and antioxidant defense status in spontaneously hypertensive rats and Wistar rats fed a diet enriched with fructose and saturated fatty acids

Aurélie Girard, M.D.[a], Sihem Madani, Ph.D.[a], Es Saddik El Boustani, Ph.D.[b], Jacques Belleville, Ph.D.[a], Josiane Prost, Ph.D.[a,*]

[a] *Université de Bourgogne, UPRES Lipides Nutrition EA 2422, Faculté des Sciences Gabriel, Dijon, France*
[b] *Nutrition et Santé, Université Caddi Ayyad, Faculté des Sciences Semlalia, Marrakech, Morocco*

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Abstract

Objective: Larger doses of fructose and saturated fat have been associated with oxidative stress and development of hypertension. The effects of modest amounts of fructose and saturated fatty acids on oxidative stress are unknown.

Methods: To increase knowledge on this question, 10-wk-old spontaneously hypertensive rats and Wistar rats were fed for 8 wk with a control diet or an experimental diet enriched with fructose (18%) and saturated fatty acids (11%; FS diet). The total antioxidant status of organs and red blood cells was assayed by monitoring the rate of free radical-induced red blood cell hemolysis. Sensitivity of very low-density lipoprotein and low-density lipoprotein (VLDL-LDL) to copper-induced lipid peroxidation was determined as the production of thiobarbituric acid-reactive substances. Antioxidant enzymes and vitamins were also measured to establish the oxidative stress effect.

Results: The FS diet did not affect blood pressure in either strain, but it increased plasma insulin concentrations only in Wistar rats without affecting those of glucose of either strain. The FS diet significantly enhanced plasma and VLDL-LDL triacylglycerol concentrations without affecting concentrations of VLDL-LDL thiobarbituric acid-reactive substances. The decreased content of arachidonic acid and total polyunsaturated fatty acids in VLDL-LDL by the FS diet may have prevented lipid peroxidation in this fraction. Moreover, FS consumption by both strains was accompanied by a significant increase in total antioxidant capacity of adipose tissue, muscle, heart, and liver. This may have resulted from increased tissue ascorbic acid levels and glutathione peroxidase and glutathione reductase activities in tissues.

Conclusions: These findings clearly indicate that the FS diet did not alter blood pressure of spontaneously hypertensive rats and Wistar rats. The FS diet resulted in hypertriglyceridemia but increased the total antioxidant status, which may prevent lipid peroxidation in these rats.

Keywords: Fructose; Saturated fatty acids; Spontaneously hypertensive rats; Antioxidant status

Introduction

Oxidative stress is obtained when the pro-oxidant challenge overwhelms the antioxidant defenses. Oxidative stress may contribute to the initiation and maintenance of hypertension by inactivation of nitric oxide (NO), which acts as vasodilator [1,2], the generation of vasoconstrictive isoprostanes [1], and vasopressor action [3]. Treatment with antioxidants may prevent or reverse abnormalities associated with hypertension and its complications. Many studies have reported that dietary supplements such as antioxidants, vitamins, and minerals prevent or at least attenuate organic impairment originated by excess oxidative stress [4,5]. In

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* Corresponding author. Tel: +33-03-80-39-63-11; fax: +33-03-80-39-63-30.

E-mail address: jprost@u-bourgogne.fr (J. Prost).

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addition, modification in the macronutrient composition of the diet is an intervention that has been advocated by some to avoid oxidative stress, specifically, diets with decreased total fat, saturated fat, sugar, and relatively increased fiber. Clinical trials [6] that have used dietary approaches to stop hypertension have shown that a diet low in refined sugar with decreased intake of saturated fat and increased intake of fruits and vegetables decreases oxidative stress and blood pressure in hypertensive and normotensive individuals. In the same way, there is evidence that hyperlipidemia [7] and high-sugar diets [8], high-fat diets [9], or both induce oxidative stress. Larger amounts of fat and refined carbohydrate in diet also have been shown to promote hypertension and endothelial dysfunction in rats [10]. However, in this situation, the investigators used diets very high in carbohydrates (60%) or fats (20%) in animal models for hypertension studies [11–13] and to induce syndromes of insulin resistance to test hypoglycemic agents [14–16]. The role of modest amounts of carbohydrate and saturated fats on oxidative stress and blood pressure is unknown.

This study investigated the effects of a diet that combined fructose (18%) and saturated fatty acids (11%; FS diet) without distinguishing between the effects of either nutrient separately in normotensive and hypertensive rats. It is representative of the Western diet that is rich in refined sugar and saturated fat, and it appeared interesting to know the effects of such a combined diet. Fructose is a glucide that can be found in foods as a simple glucide and as a component of sucrose. Because of the use of high-fructose corn sweeteners and of sucrose in manufactured foods, the dietary consumption of fructose has increased several-fold from that present in natural food [17]. In the present study, the consequence of this FS diet in rats was assessed by measuring several variables related to lipid metabolism, blood pressure, and oxidative stress in blood and tissues.

Materials and methods

Animals and diets

Ten-week-old male, spontaneously hypertensive rats (SHRs; $n = 12$) and Wistar rats ($n = 12$) were purchased from Janvier (Le Ginestet St Isle, France). They were maintained at 24°C and constant humidity (60%) with a 12-h light, 12-h dark cycle. Each group of rats was divided into two groups of six rats each and fed different diets for 8 wk. One group received the control diet. The other group received the FS diet (Table 1). Sucrose and some starch were substituted with fructose and saturated fat in the FS diet. Food and tap water were freely available. Levels of selected vitamins and mineral salt were in line with recommended requirements [18]. We followed the general guidelines for the care and use of laboratory animals recommended by the Council of European Communities.

Table 1
Composition of the diets

Constituents (g/kg diet)	Control diet	FS diet
Casein*	260	260
Methionine†	3	3
Starch*	549.3	299.3
Sucrose*	44	—
Fructose*	—	184
Cellulose*	50	50
Vitamin mix‡	3	3
Mineral mix§	40	40
Isio 4‖	50	50
Végétaline‖	—	110
Choline chloride (98%)†	0.7	0.7

Végétaline provided the following fatty acids (% total fatty acids): C6:0, 0.53; C8:0, 7.57; C10:0, 6.02; C12:0, 47.87; C14:0, 18.19; C16:0, 9.27; C18:0, 9.65; C18:1, 0.90.

FS, enriched with fructose and saturated fatty acids

* Purchased from UAR (Villemoisson, Epinay sur Orge, France).

† Purchased from Prolabo (Paris, France).

‡ Purchased from UAR 200 (Villemoisson). This vitamin mix provided the following nutrients (mg/kg of dry diet): retinol, 1.8; cholecalciferol, 0.019; thiamine, 6; riboflavin, 4.5; pantothenic acid, 21; pyridoxine, 3; inositol, 45; cyanocobalamin, 0.015; ascorbic acid, 240; DL-α-tocopherol, 51; menadione, 12; nicotinic acid, 30; para-aminobenzoic acid, 15; folic acid, 1.5; biotin, 0.09.

§ Purchased from UAR 205B (Villemoisson). This mineral mix provided the following nutrients (g/kg of dry diet): Ca, 4; K, 2.4; Na, 1.6; Mg, 0.4; Fe, 0.12; elements (traces): Mn, 0.032; Cu, 0.005; Zn, 0.018; Co, 0.00004; I, 0.00002, completed to 40 000 with cellulose.

‖ Commercial products. Fatty acid compositions of Isio 4 expressed as percentages: C16:0, 6.1; C16:1 (ω-7), 0.1; C18:0, 3.63; C20:0, 0.3; C22:0, 0.7; C18:1 (ω-9), 38.6; C20:1 (ω-9), 0.23; C18:2 (ω-6), 44.73; C18:3 (ω-3), 1.3. Total saturated fatty acids: 10.8; total monounsaturated fatty acids: 40.2; total polyunsaturated fatty acids (ω-6): 47.2; total polyunsaturated fatty acids (ω-3): 1.7; total saturated fatty acids/total monounsaturated fatty acids; 0.30; total saturated fatty acids/total polyunsaturated fatty acids (ω-6/ω-3): 0.22; total polyunsaturated fatty acids (ω-6)/total polyunsaturated fatty acids (ω-3): 27.8.

Blood pressure measurement

Systolic blood pressure of conscious rats after 4 and 8 wk on the diets was measured by a non-bloody tail-cuff method [19]. Blood pressure values are the means of at least four measurements per rat, and only measurements at 8 wk are reported in Table 2.

Analytical procedures

Blood samples and tissue preparation

After the 8-wk dietary period, rats were deprived of food for 12 h and then anesthetized with sodium pentobarbital (60 mg/kg of body weight). Blood was collected from the abdominal aorta into tubes containing ethylene-diaminetetraacetic acid, and plasma was prepared by low-speed centrifugation (1000*g* for 20 min). Plasma concentrations of total cholesterol, triacylglycerol, and phospholipid were determined with enzyme kits (Boehringer, Meylan, France). Fasting glycemia was determined with glucometer (Life-

Table 2
Food intake, BW, relative organ weights, blood pressure, glycemia, and insulinemia in SHR and WSR fed control and FS diets*

	WSR-C	WSR-FS	SHR-C	SHR-FS
Initial BW (g)	320 ± 29	339 ± 18	259 ± 14‡	260 ± 24‡
Final BW (g)	491 ± 40	488 ± 33	338 ± 20‡	344 ± 35‡
Food intake (g/d)	19.2 ± 2.9	20.3 ± 3.5	18.8 ± 3.1	19.5 ± 2.6
Relative liver weight (g/100 g BW)	2.05 ± 0.28	2.11 ± 0.14	2.57 ± 0.15‡	2.44 ± 0.16‡
Relative kidney weight (g/100 g BW)	0.65 ± 0.05	0.61 ± 0.02	0.74 ± 0.03‡	0.73 ± 0.05‡
Relative heart weight (g/100 g BW)	0.34 ± 0.07	0.29 ± 0.04	0.44 ± 0.03‡	0.45 ± 0.05‡
Glycemia (g/L)	0.86 ± 0.05	0.95 ± 0.05	1.18 ± 0.10‡	1.12 ± 0.08‡
Insulinemia (pM/L)	572 ± 78	709 ± 69†	796 ± 90‡	733 ± 26‡
Blood pressure at 8 wk (mmHg)	139 ± 8	138 ± 10	213 ± 8‡	206 ± 6‡

BW, body weight; FS, enriched with fructose and saturated fatty acids; SHR, spontaneously hypertensive rats; SHR-C, SHR fed the control diet; SHR-FS, SHR-fed the FS diet; WSR, Wistar rats; WSR-C, Wistar rats fed the control diet; WSR-FS, Wistar rats fed the FS diet

* Values are mean ± standard deviation ($n = 6$ rats/group).

† $P < 0.05$, FS versus control diet regardless of strain.

‡ $P < 0.05$, SHR versus WSR regardless of diet.

scan, Issy-les-Moulineaux, France). Insulin was measured with enzyme-linked immunosorbent assay, and bovine insulin was used as a standard, anti-bovine insulin as a primary antibody, and peroxidase-conjugated anti–immunoglobulin G as a secondary antibody. Absorbance was measured at 492 nm. Plasma lipids were extracted according to the method of Folch et al. [20]. After adding heptadecanoic acid (used as an internal standard), total lipids of plasma were saponified and methylated according to the method of Slover and Lanza [21] and then analyzed by capillary gas-liquid chromatography (Becker Packard model 417 gas-liquid chromatograph equipped with a Spirawax column: Carbowax 20 M, 30 m × 0.3 mm inner diameter, 0.1 μm thick; Spiral RD, Couternon, France) at a constant temperature of 190°C, with a helium flow rate of 6 mL/min. The detector response was checked with a standard mixture of methyl esters (Nu-Chek-Prep, Elysian, MN, USA).

Liver, heart, kidney, adipose tissues, muscle, and brain were removed immediately, rinsed with cold saline, and weighed. Liver lipids were extracted according to the method of Folch et al. [20]. Liver total cholesterol, triacylglycerol, and phospholipid were determined with enzyme kits (Biomerieux, Lyon, France). Proteins were estimated according to the method of Schacterle and Pollack [22] with bovine serum albumin as a standard.

α-Tocopherol and ascorbic acid determination

Plasma α-tocopherol was determined by high-performance liquid chromatography with a Varian system after hexanic extraction and tocol (Lara Spiral, Couternon, France) as an internal standard. The chromatographic conditions were a 3.6- × 250-mm column, C18 symmetry (Supelcosil, Supelco-Sigma-Aldrich, L'Ilsle D'Abeau Chesne, France), 20-μL injection volume, mobile-phase methanol:water (98:2, v/v), 1.5-mL/min flow rate, 8 min retention time, and detection at 285 nm. Plasma, liver, kidney, and heart ascorbic acid amounts were measured by the method of Roe and Kueter [23].

NO and glutathione determination

NO determination was performed by using the Griess reagent (sulfanilamide and *n*-naphthyl-ethylene diamine) [24]. Plasma and tissue extracts were clarified by zinc sulfate solution, and NO_3 was then reduced to NO_2 by cadmium overnight at 20°C under shaking. Samples were added to the Griess reagent and incubated for 20 min at room temperature. Absorbance was measured at 540 nm. Sodium nitrite was used for a standard curve.

Total glutathione in erythrocytes and organs was measured according to the procedure of Anderson [25] by using reduced glutathione as a standard.

Lipid peroxidation

The fraction of very low-density lipoprotein and low-density lipoprotein (VLDL-LDL) obtained by precipitation with dextran sulfate (0.6 g/L) and $MgCl_2$ (0.91 M/L) [20] was used. As described by Frémont et al. [26], 100 μL (250 μg of protein) of VLDL-LDL fraction was added to 900 μL of phosphate buffer (10 mM/L) and incubated in duplicate for 24 h at 37°C with 20 μL of $CuSO_4$ (0.25 mM/L). Free radical damage was determined by specifically measuring thiobarbituric acid-reactive substances (TBARS), as described by Quintanilha et al. [27]. TBARS were determined by the spectrophotometric method using malondialdehyde and prepared by tetrahydroxypropane hydrolysis to establish the standard curve. Results are expressed as nanomoles of TBARS per milliliter of plasma.

Red blood cell susceptibility to hemolysis

Resistance to free radical aggression was tested as the capacity of red blood cells (RBCs) to withstand free radical-induced hemolysis and was measured, as described by Blache and Prost [28], by monitoring the rate of free radical-induced hemolysis with a microplate titrator (iEMS Reader MF, Kirial SA, Couternon, France). Blache and Prost [28] clearly demonstrated that, if at least one component of the antiradical detoxification system (antioxidants, enzymes) is impaired, a shift of the hemolysis curve is obtained toward shorter times.

Briefly, washed RBCs were diluted (1:40, v/v) with KRL buffer (300 mosm/L) and 50 μL of RBC suspension was assayed in a 96-well microplate coated with a free radical generator (GRL, Kirial SA). The kinetic of RBC resistance to hemolysis was determined at 37°C by continuous monitoring of changes (620 nm absorbance). The time to reach 50% of total hemolysis was retained for group comparisons.

Total antioxidant capacity of organs

The synergic effect of antioxidants in organs provides greater protection against free radical attacks than any antioxidant alone. Therefore, determining the total antioxidant capacity of organs is of great interest because it allows assessment of the capacity of the system to withstand oxidative stress [25]. Organs (liver, kidney, heart, muscle, brain, and adipose tissue) were washed three times in 150 mM/L of NaCl and homogenized in a Potter Elvehjhem Tissue Homogenizer (VWR International, Fontenay-sous-Bois, France) with KRL buffer (100 mg/5 mL), and then homogenates were sonicated for 5 min and centrifuged for 20 min at 5000*g*. A 96-well microplate coated with a free radical generator (Kirial SA) was rehydrated with KRL buffer (170 μL), and 50 μL of organ homogenate (supernatant) was added to 50 μL of control RBCs. Control RBCs from donor rats were prepared under the same conditions as the experimental RBCs. The kinetic of hemolysis was determined as described above.

Antioxidant enzyme measurements

Enzyme activity in tissue was expressed as units per milligram of protein. All enzyme activities were adapted to microplate titration with the microplate titrator iEMS Reader MF. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured at 412 nm by testing the inhibition degree of nitrite formation [29]. SOD activity was compared with those of standard solutions of known activity. Glutathione peroxidase (GSH-Px; EC 1.11.1.9) was determined according to the method of Paglia and Valentine [30] using cumene hydroperoxide as a substrate. One unit of GSH-Px was defined as the oxidation by H_2O_2 of 1 μM of reduced glutathione per minute at pH 7 and 25°C. Glutathione reductase (GSSG-Red; EC 1.6.4.2) activity was evaluated at 340 nm by measuring the decrease in absorbance of nicotinamide adenine dinucleotide phosphate (reduced) in the presence of oxidized glutathione [31]. One unit of enzyme reduced 1 μM of oxidized glutathione per minute at pH 7.6 and 25°C.

Statistical analysis

Data were reported as means ± standard deviation for six rats per group. Statistical analysis of the data was carried out with STATISTICA 4.1 (Statsoft, Tulsa, OK, USA). Data were tested by one-way analysis of variance. A difference of $P < 0.05$ was considered statistically significant.

Results

The control diet was used to assess the effects of the FS diet. When the strain effects were determined, only the control diet was considered.

Food consumption, body and organ weights, blood pressure, and plasma glucose and insulin concentrations

Strain effect

Food intake did not differ between groups (Table 2). Body weights at the start and at the end of the experiment were significantly lower in SHRs than in Wistar rats. However, liver, kidney, and heart relative weights, systolic blood pressure, and plasma glucose and insulin concentrations were significantly higher in the SHR group than in the Wistar group.

Diet effect

Feeding the FS diet did not affect blood pressure, normal growth, or plasma glucose concentrations but did significantly enhance plasma insulin levels only in Wistar rats (Table 2).

Liver, plasma, and lipoprotein lipid concentrations

Strain effect

Liver, plasma, and lipoprotein cholesterol and phospholipid concentrations were similar in SHRs and Wistar rats (Table 3). However, triacylglycerol levels in the liver were significantly higher in SHRs than in Wistar rats.

Diet effect

Compared with the control diet, the FS diet did not affect liver plasma and lipoprotein cholesterol and phospholipid concentrations but did significantly enhance plasma and VLDL-LDL triacylglycerol concentrations in both strains (Table 3).

TBARS concentrations, RBC resistance, and total antioxidant capacity of organs

Strain effect

SHRs versus Wistar rats exhibited higher concentrations of VLDL-LDL TBARS (Table 4). The total antioxidant capacity of the heart was higher and that of muscle was lower in SHRs than in Wistar rats. The total antioxidant capacities of erythrocytes, liver, kidney, adipose tissue, and brain were not modified between the two groups of rats.

Diet effect

Concentrations of VLDL-LDL TBARS and RBC resistance against free radical attack were not affected by either diet (Table 4). However, the FS diet increased the total antioxidant capacity of the liver, heart, adipose tissue, and muscle in SHRs and Wistar rats.

Table 3
Liver, plasma, and lipoprotein total cholesterol, triacylglycerol, and phospholipid concentrations in SHR and WSR fed control and FS diets*

	WSR-C	WSR-FS	SHR-C	SHR-FS
Total cholesterol				
Liver (μM/g)	2.55 ± 0.56	3.26 ± 1.43	3.18 ± 1.32	3.90 ± 2.63
Plasma (mM/L)	3.26 ± 0.67	3.24 ± 0.54	2.97 ± 0.35	2.79 ± 0.59
VLDL-LDL (mM/L)	1.51 ± 0.51	1.60 ± 0.33	1.24 ± 0.26	0.85 ± 0.42
HDL (mM/L)	1.75 ± 0.26	1.64 ± 0.24	1.73 ± 0.28	1.94 ± 0.49
Triacylglycerol				
Liver (μM/g)	10.4 ± 0.58	10.8 ± 3.0	15.59 ± 2.5‡	19.6 ± 5.5‡
Plasma (mM/L)	0.87 ± 0.19	1.60 ± 0.52†	0.58 ± 0.18	1.14 ± 0.49†
VLDL-LDL (mM/L)	0.41 ± 0.06	0.84 ± 0.38†	0.39 ± 0.05	0.63 ± 0.20†
Phospholipids				
Liver (μM/g)	13.4 ± 2.1	17.0 ± 7.0	14.2 ± 5.7	17.7 ± 12.2
Plasma (mM/L)	1.90 ± 0.26	1.62 ± 0.26	1.64 ± 0.32	1.72 ± 0.30
VLDL-LDL (mM/L)	0.94 ± 0.23	0.79 ± 0.17	0.89 ± 0.23	0.87 ± 0.19
HDL (mM/L)	0.95 ± 0.10	0.83 ± 0.11	0.85 ± 0.30	0.85 ± 0.22

FS, enriched with fructose and saturated fatty acids; HDL, high-density lipoprotein; SHR, spontaneously hypertensive rats; SHR-C, SHR fed the control diet; SHR-FS, SHR fed the FS diet; VLDL-LDL, very low-density lipoprotein and low-density lipoprotein; WSR, Wistar rats; WSR-C, Wistar rats fed the control diet; WSR-FS, Wistar rats fed the FS diet

* Values are mean ± standard deviation ($n = 6$ rats/group).

† $P < 0.05$, FS versus control diet regardless of strain.

‡ $P < 0.05$, SHR versus WSR regardless of diet.

Antioxidant enzyme activities in erythrocytes, liver, heart, kidney, and adipose tissue

Strain effect

SHRs versus Wistar rats fed the control diet exhibited enhanced activity of SOD in muscle, GSH-Px in heart, and GSSG-Red in heart, adipose tissue, and muscle (Table 5).

Diet effects

Compared with the control diet, the FS diet did not affect SOD activity in any tissue, whatever the strain, except in brain, where SOD was significantly higher in Wistar rats fed the FS diet instead of the control diet (Table 5). The FS diet increased erythrocyte glutathione concentrations only in Wistar rats (Table 5) and did not affect those of organs, whatever the strain (results not shown). GSH-Px and GSSG-Red enzyme activities were markedly affected by the FS diet in Wistar rats but were lower in SHRs. Wistar rats fed the FS diet exhibited enhanced both enzyme activities in all tissues, except liver, kidney, and adipose tissue for GSH-Px and brain for GSSG-Red. SHRs fed the FS diet exhibited enhanced GSH-Px activity only in adipose tissue and brain.

Plasma α-Tocopherol, NO, and plasma and tissue concentrations of ascorbic acid

Strain effects

Plasma concentrations of ascorbic acid were higher, whereas those of α-tocopherol were lower in SHRs than in Wistar rats (Table 6). No significant differences in NO concentrations in plasma were found between the two groups of rats.

Table 4
VLDL-LDL TBARS concentrations, susceptibility of RBC to hemolysis, and total antioxidant capacity of organs in SHR and WSR fed control and FS diets*

	WSR-C	WSR-FS	SHR-C	SHR-FS
VLDL-LDL TBARS (nM/mL plasma)	0.031 ± 0.007	0.035 ± 0.011	0.055 ± 0.015‡	0.056 ± 0.005‡
RBC resistance, time to reach 50% hemolysis (min)	72.7 ± 11.6	66.3 ± 6.7	64.8 ± 3.7	65.7 ± 5.4
Liver, time to reach 50% hemolysis (min)	35.5 ± 2.8	36.8 ± 2.1	29.3 ± 5.6	34.9 ± 1.6†
Kidney, time to reach 50% hemolysis (min)	67.9 ± 6.1	70.5 ± 4.2	71.8 ± 2.0	70.4 ± 3.5
Heart, time to reach 50% hemolysis (min)	92.9 ± 8.1	105.4 ± 5.6†	111.9 ± 2.7‡	117.6 ± 8.8†‡
Adipose tissue, time to reach 50% hemolysis (min)	51.5 ± 3.3	60.5 ± 3.2†	48.2 ± 3.5	58.9 ± 6.4†
Muscle, time to reach 50% hemolysis (min)	65.1 ± 2.4	69.7 ± 2.5†	60.3 ± 2.4‡	65.5 ± 1.8†‡
Brain, time to reach 50% hemolysis (min)	63.9 ± 2.3	62.8 ± 1.2	63.8 ± 2.3	64.4 ± 2.0

FS, enriched with fructose and saturated fatty acids; SHR, spontaneously hypertensive rats; SHR-C, SHR fed the control diet; SHR-FS, SHR fed the FS diet; RBC, red blood cell; TBARS, thiobarbituric acid-reactive substances; WSR, Wistar rats; WSR-C, Wistar rats fed the control diet; VLDL-LDL, very low-density lipoprotein and low-density lipoprotein; WSR-FS, Wistar rats fed the FS diet

* Values are mean ± standard deviation ($n = 6$ rats/group).

† $P < 0.05$, FS versus control diet regardless of strain.

‡ $P < 0.05$, SHR versus WSR regardless of diet.

Table 5
Erythrocyte GSH concentrations, and antioxidant enzyme activities in erythrocytes and organs in SHR and WSR fed control and FS diets*

	WSR-C	WSR-FS	SHR-C	SHR-FS
Erythrocytes				
GSH (μM/L)	0.37 ± 0.09	0.51 ± 0.14†	0.24 ± 0.12	0.31 ± 0.15‡
SOD (U/mL)	243.9 ± 32.3	274.4 ± 48.9	275.7 ± 13.9	271.6 ± 8.2
GSH-Px (U/g Hb)	51.1 ± 18.8	72.3 ± 15.5†	56.1 ± 7.7	42.1 ± 13.2‡
GSSG-Red (U/g Hb)	37.1 ± 16.9	59.8 ± 21.2†	45.0 ± 17.7	28.3 ± 10.5‡
Liver (U/mg protein)				
SOD	42.9 ± 9.5	41.6 ± 4.3	41.1 ± 9.2	44.9 ± 9.5
GSH-Px	12.4 ± 1.5	11.7 ± 1.0	13.5 ± 3.6	13.4 ± 2.5
GSSG-Red	75.0 ± 2.5	91.5 ± 1.9†	91.4 ± 17.5	94.5 ± 16.1
Kidney (U/mg protein)				
SOD	29.7 ± 7.7	31.7 ± 4.4	26.5 ± 3.3	29.1 ± 1.3
GSH-Px	21.3 ± 5.2	22.3 ± 6.1	20.5 ± 1.7	21.5 ± 3.4
GSSG-Red	50.9 ± 8.5	66.3 ± 2.6†	65.9 ± 6.5	67.5 ± 9.2
Heart (U/mg protein)				
SOD	2.23 ± 0.99	3.09 ± 0.34	2.80 ± 0.45	2.21 ± 0.78
GSH-Px	57.8 ± 14.0	112.0 ± 6.4†	104.3 ± 11.9‡	95.1 ± 31.7
GSSG-Red	6.9 ± 1.3	11.9 ± 1.5†	12.7 ± 1.0‡	10.5 ± 3.8
Adipose tissue (U/mg protein)				
SOD	48.7 ± 14.1	49.8 ± 12.3	51.2 ± 14.3	48.0 ± 12.5
GSH-Px	8.56 ± 2.99	6.92 ± 1.76	7.62 ± 1.71	14.45 ± 4.72‡
GSSG-Red	2.49 ± 0.73	4.06 ± 0.66†	4.36 ± 1.27‡	5.57 ± 1.16‡
Muscle (U/mg protein)				
SOD	4.06 ± 0.59	4.47 ± 1.92	8.87 ± 2.05‡	9.47 ± 1.71‡
GSH-Px	150.0 ± 14.0	223.0 ± 16.0†	210 ± 45	181 ± 40
GSSG-Red	57.2 ± 9.6	72.7 ± 7.8†	76.7 ± 17.1‡	79.2 ± 6.1
Brain (U/mg protein)				
SOD	0.09 ± 0.02	0.12 ± 0.01†	0.08 ± 0.02	0.07 ± 0.03
GSH-Px	281.0 ± 33.0	324.0 ± 10.0†	308.0 ± 16.0	352.0 ± 24.0†
GSSG-Red	243 ± 29	225 ± 20	266 ± 29	248 ± 33

FS, enriched with fructose and saturated fatty acids; GSH-Px, glutathione peroxidase; GSSG-Red, glutathione reductase; Hb, hemoglobin; SHR, spontaneously hypertensive rats; SHR-C, SHR fed the control diet; SHR-FS, SHR fed the FS diet; RBC, red blood cell; SOD, superoxide dismutase; WSR, Wistar rats; WSR-C, Wistar rats fed the control diet; WSR-FS, Wistar rats fed the FS diet

* Values are mean ± standard deviation ($n = 6$ rats/group).

† $P < 0.05$, FS versus control diet regardless of strain.

‡ $P < 0.05$, SHR versus WSR regardless of diet.

Diet effect

When fed the FS diet, only SHRs exhibited significant higher plasma NO concentrations (Table 6). In organs, however, no significant differences in NO concentrations were produced by either diet (results not shown). Plasma concentrations of α-tocopherol were not affected by diet. Compared

Table 6
Nitric oxide and α-tocopherol and ascorbic acid concentrations in plasma and tissues of SHR and WSR fed control and FS diets*

	WSR-C	WSR-FS	SHR-C	SHR-FS
Nitric oxide				
Plasma (nM/L)	28.4 ± 10.7	26.0 ± 8.7	28.7 ± 4.7	46.2 ± 7.9†
α-Tocopherol				
Plasma (μg/mL)	8.7 ± 1.5	7.9 ± 1.6	5.3 ± 1.1‡	5.5 ± 2.7‡
Ascorbic acid				
Plasma (μg/mL)	17.6 ± 4.5	19.0 ± 3.1	27.4 ± 3.5‡	47.1 ± 7.9†‡
Liver (μg/g)	229.0 ± 38.0	352.0 ± 41†	248.4 ± 47.0	352.4 ± 40.9†
Kidney (μg/g)	91.5 ± 8.0	104.5 ± 7.9†	98.8 ± 7.5	102.3 ± 3.5
Heart (μg/g)	119.7 ± 33.6	102.9 ± 13.3	118.2 ± 31.9	119.6 ± 42.9

FS, enriched with fructose and saturated fatty acids; SHR, spontaneously hypertensive rats; SHR-C, SHR fed the control diet; SHR-FS, SHR fed the FS diet; WSR, Wistar rats; WSR-C, Wistar rats fed the control diet; WSR-FS, Wistar rats fed the FS diet

* Values are mean ± standard deviation ($n = 6$ rats/group).

† $P < 0.05$, FS versus control diet regardless of strain.

‡ $P < 0.05$, SHR versus WSR regardless of diet.

Table 7
Fatty acid composition of VLDL-LDL lipids of SHR and WSR fed control and FS diets*

Composition (g/100 g fatty acids)	WSR-C	WSR-FS	SHR-C	SHR-FS
16:0	27.9 ± 4.0	29.0 ± 4.1	22.8 ± 1.9‡	28.7 ± 5.9
18:0	16.6 ± 5.1	16.6 ± 1.3	12.8 ± 0.8‡	18.6 ± 1.3†
18:1	16.5 ± 1.2	20.1 ± 3.1	15.3 ± 1.2	20.8 ± 2.5†
18:2ω-6	13.2 ± 2.5	12.5 ± 2.7	14.8 ± 1.6	12.2 ± 2.1
20:4ω-6	25.8 ± 7.1	21.8 ± 5.2	34.3 ± 3.1‡	19.4 ± 4.2†
ΣPUFA	39.0 ± 8.9	34.4 ± 6.8	47.6 ± 4.0‡	30.6 ± 5.9†

FS, enriched with fructose and saturated fatty acids; ΣPUFA, total polyunsaturated fatty acids; SHR, spontaneously hypertensive rats; SHR-C, SHR fed the control diet; SHR-FS, SHR fed the FS diet; VLDL-LDL, very low-density lipoprotein and low-density lipoprotein; WSR, Wistar rats; WSR-C, Wistar rats fed the control diet; WSR-FS, Wistar rats fed the FS diet

* Values are mean ± standard deviation ($n = 6$ rats/group).

† $P < 0.05$, FS versus control diet regardless of strain.

‡ $P < 0.05$, SHR versus WSR regardless of diet.

with the control diet, the FS diet increased levels of ascorbic acid in the liver and kidney of Wistar rats and in plasma and liver of SHRs.

VLDL-LDL fatty acid composition

Strain effects

SHRs exhibited significantly lower levels of 16:0 and 18:0 but higher levels of 20:4ω-6 and total polyunsaturated fatty acids (PUFA) than did Wistar rats (Table 7).

Diet effect

The fatty acid composition of VLDL-LDL (Table 7) did not differ greatly between groups fed the control diet and the FS diet. The main differences were observed in levels of 18:0 and 18:1, which were significantly higher, whereas those of 20:4ω-6 and PUFA were lower with the FS diet than with the control diet, whatever the strain.

Discussion

Several studies have reported that a diet high in saturated fat and sucrose promotes hypertension and endothelial dysfunction and induces marked oxidative stress in Wistar rats [32]. However, these investigators used diets very high in carbohydrate (45%) and fat (20%) in animal models to induce insulin resistance, with metabolic changes similar to those observed in syndrome X, a disorder in which a high incidence of cardiovascular disease has been described. Whether modest amounts of saturated fat and sugar could influence blood pressure and antioxidant status is unknown. In this study, we investigated the effect of a diet enriched with fructose (18%) and saturated fatty acids (11%) on lipid metabolism, blood pressure, and antioxidant defense status in normotensive (Wistar) and hypertensive (SHR) rats.

SHRs were significantly hyperglycemic and hyperinsulinemic compared with Wistar rats, indicating insulin resistance. There is evidence that insulin resistance is positively correlated to glycemia and insulinemia [33]. Another study demonstrated insulin resistance in this strain [33]. The FS diet compared with the control diet increased plasma insulin concentrations only in the Wistar rats, whereas plasma glucose concentrations were unchanged in either strain, suggesting no dietary effect on insulin resistance in either strain. This may explain the constant blood pressure observed with the FS diet in SHRs and Wistar rats. Previous reports have demonstrated that a diet high in refined sugar increases arterial blood pressure in rats and human [32], and this increase was attributed to a decrease in insulin sensitivity induced by this diet. Roberts et al. [32] investigated high levels of sugar and fat (corn oil) in the diet and reported increased blood pressure in normal rats. In the present study, the increase in blood pressure by fructose may have been prevented by saturated fat. Dietary saturated fat (butterfat) compared with corn oil, which is highly unsaturated, has been shown to lower blood pressure in SHRs [34]. Conversely, plasma NO concentrations (potent vasodilating substance) were increased by the FS diet only in SHRs. This could have been mediated by the significant increase in plasma and liver concentrations of ascorbic acid in this strain (Table 6), which may activate the NO system [35]. However, the increase in NO concentrations with the FS diet was not associated with a decrease in blood pressure in these rats, indicating factors other than NO in the modulation of blood pressure with the FS diet.

Despite a similar food intake, the SHRs had lower body weight gains and final body weights compared with Wistar rats. These observations are qualitatively similar to those reported by Swislocki and Tsuzuki [33]. However, relative weights of liver, kidney, and heart were significantly greater in the SHR group than in the Wistar group. These results suggest that the development of visceral obesity in these rats is likely due to increased synthesis of protein and triacylglycerols (as shown in the livers of SHR versus Wistar group; Table 3). Several reports have demonstrated that insulin stimulates lipogenesis and protein synthesis [36,37]. This anabolic action of insulin was shown to result from

signal transduction through a rapamycin-sensitive pathway [38,39]. The FS diet affected neither normal growth nor liver lipid concentrations but significantly increased plasma and VLDL-LDL triacylglycerol concentrations in both strains. This observation is consistent with those of previous reports that have demonstrated hypertriglyceridemia after feeding diets enriched with fructose and saturated fatty acid [40,41]. Hypertriglyceridemia may be secondary to increases in the VLDL triacylglycerol secretion rate because elevations in plasma triacylglycerol levels have been correlated with increases in this rate [42,43]. Previous studies have shown that the VLDL triacylglycerol secretion rate in livers of rats fed a diet high in sucrose lard or fructose lard is higher than that of controls [42,43]. Further, decreased tissue lipoprotein lipase activity and impaired clearance of triacylglycerols have been reported in rats fed a diet high in sucrose and fat [44].

SHRs had higher concentrations of VLDL-LDL TBARS (Table 4) than did Wistar rats (Table 6). This could have resulted from the larger proportions of total PUFA and 20:4ω-6 in the VLDL-LDL fraction. The production of conjugated dienes and that of TBARS in the VLDL-LDL fraction were shown to depend mainly on the relative content of PUFA and particularly that of 20:4ω-6, which is considered one of the most prone to oxidation [11,45–47]. Enhanced superoxide anion production has been demonstrated in stroke-prone SHRs compared with Wistar Kyoto rats [48]. This may partly explain the lower concentrations of plasma α-tocopherol and higher concentrations of ascorbic acid in SHRs (Table 6) because α-tocopherol is used to a larger extent to scavenge free radicals, and ascorbic acid is used to regenerate α-tocopherol. Hence, the increased values of plasma ascorbic acid in SHRs compared with those of Wistar rats could result from an increased endogenous synthesis in response to oxidative stress in these rats. The FS diet did not affect levels of VLDL-LDL TBARS in either strain, indicating no apparent increase in free radical generation in this lipoprotein fraction. Several other studies have shown that sucrose or fructose produce an increase in VLDL triacylglycerol associated with a decrease in plasma vitamin E and increased lipid peroxidation [32]. Vitamin E depletion in sucrose- or fructose-fed rats predispose VLDL and LDL enriched with triacylglycerol to subsequent oxidative stress [49], which is one of the critical mechanisms involved in the progression of atherosclerosis. In the present study, the FS diet did not affect plasma vitamin E concentrations but did increase (particularly in liver) those of ascorbic acid, also considered an antioxidant agent in SHRs and Wistar rats. These findings may explain the unchanged concentrations of VLDL-LDL TBARS with the FS diet. Further, the decreased levels of PUFA and 20:4ω-6 in the VLDL-LDL fraction (Table 7) might have prevented lipid peroxidation in rats fed the FS diet despite increased triacylglycerol concentrations.

In our study, we also demonstrated that the FS diet in both strains led to a significant increase in the total antioxidant capacity of liver, heart, adipose tissue, and muscle (Table 4). Changes in tissue levels of ascorbic acid and antioxidant enzyme activities could provide an explanation for these findings. The positive correlation between total antioxidant capacity, ascorbic acid levels, and GSH-Px and GSSG-Red activities in liver, heart, adipose tissue, and muscle (Tables 4 to 6) suggests that the increase in total antioxidant capacity of a tissue is linked to the increased ascorbic acid concentrations and/or to the increased antioxidant enzyme activities, which are favorable factors to check the generation of free radical and lipid hydroperoxides [50].

Together these findings clearly indicate that the FS diet did not alter blood pressure and antioxidant status of either strain, but significantly increased plasma triacylglycerol concentrations, which comprise a risk factor for cardiovascular diseases. Thus, diets high in fructose and saturated fat may be undesirable for men.

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