Metabolic Syndrome in the Rat: Females Are Protected Against the Pro-Oxidant Effect of a High Sucrose Diet

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Metabolic syndrome is more prevalent in men than in women. In an experimental dietary model of metabolic syndrome, the high-fructose–fed rat, oxidative stress has been observed in males. Given that estradiol has been documented to exert an antioxidant effect, we investigated whether female rats were better protected than males against the adverse effects of a high-sucrose diet, and we studied the influence of hormonal status in female rats. Males and females were first fed a sucrose-based or starch-based diet for 2 weeks. In the males, the plasma triglyceride (TG)-raising effect of sucrose was accompanied by significantly lowered plasma α-tocopherol and a significantly lowered α-tocopherol/TG ratio (30%), suggesting that vitamin E depletion may predispose lipoproteins to subsequent oxidative stress. In males, after exposure of heart tissue homogenate to iron-induced lipid peroxidation, thiobarbituric reactive substances were significantly higher in the sucrose-fed than in the starch-fed rats. In contrast, in sucrose-fed females, neither a decrease in vitamin E/TG ratio nor an increased susceptibility of heart tissue to peroxidation was observed, despite both a significantly decreased heart superoxide dismutase activity (14%) and a significant 3-fold increase in plasma nitric oxide concentration compared with starch-fed females. The influence of hormonal status in female rats was then assessed using intact, ovariectomized, or estradiol-supplemented ovariectomized female rats fed the sucrose or starch diet for 2 weeks. After exposure of heart tissue to iron-induced lipid peroxidation, higher susceptibility to peroxidation was found only in ovariectomized females fed the sucrose diet compared with the starch group and not in intact females or ovariectomized females supplemented with estradiol. Thus, estrogens, by their effects on antioxidant capacity, might explain the sexual difference in the **pro-oxidant effect of sucrose diet resulting in metabolic syndrome in rats.** Exp Biol Med 227:837–842, 2002

Key words: metabolic syndrome; high-sucrose diet; oxidative stress; gender; rat.

A cluster of abnormalities, including among other signs, hypertension, insulin resistance, and dyslipidemia, has been designated syndrome X or metabolic syndrome (1). Its prevalence, based on the 1998 WHO definition (2), has been estimated to be higher in men than in women (3). Although genetic influence is undoubtedly a strong factor in predisposition to insulin resistance, diet also plays a significant role (4). D-fructose occurs in foods both as a simple sugar and as a component of the disaccharide sucrose composed of one molecule of glucose and one of fructose. Although there is little evidence that modest amounts of fructose have detrimental effects on carbohydrate and lipid metabolisms, larger doses of fructose have been associated with numerous metabolic abnormalities in humans and laboratory animals (5, 6). Healthy subjects who received 17% of their energy intake in the form of fructose for 6 weeks showed increased fasting and postprandial plasma triacylglycerol concentrations (7). In addition, ≥30% of total energy intake as sucrose is thought to have an adverse effect on insulin sensitivity (8). Because of the use of high-fructose corn sweeteners and of sucrose in manufactured foods, the dietary consumption of fructose is now several times higher than that provided by natural foods (8). The 1990 U.K. national dietary and nutritional survey showed that a substantial number of apparently healthy people obtain >30% of their total energy from non-milk sugar (predominantly sucrose), representing intakes for which adverse effects on insulin sensitivity may occur (8, 9). Data from a recent investigation that evaluated the food sources of added sweeteners in the U.S. diet are consistent with these observations and suggest that the percentage of fructose in the diet is still rising (10). High-sucrose

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and high-fructose diets can be used in animal models to induce the metabolic changes observed in syndrome X (11, 12). The underlying mechanisms for the detrimental consequences of a high-fructose diet in animal model are not clear, but fructose feeding may facilitate oxidative damage (13). We have recently demonstrated that in weaning rats, a short-term high-sucrose diet has a pro-oxidant effect (14, 15) that may contribute to the etiology of syndrome X. Because estradiol has been documented as exerting antioxidant effects (16, 17) and given that female rats do not develop sucrose-induced insulin resistance (18), we conducted two experiments to determine whether female rats were protected against the pro-oxidant effect of a high-sucrose diet. In the first experiment, we assessed the effect of a high-sucrose diet versus starch diet on oxidative stress parameters in male and female rats. A menopausal metabolic syndrome, originating from estrogen deficiency and restored by estrogen replacement, has been suggested as a risk factor for coronary heart diseases (19). A second study was therefore performed to assess the effect of hormonal status on heart susceptibility to lipid peroxidation in female rats, using intact, ovariectomized or estradiol-supplemented ovariectomized animals.

Materials and Methods

Experimental Design. Male and female Wistar-Han rats from a laboratory animal colony (INRA: National Institute of Agricultural Research, Clermont-Ferrand, France) were housed in wired-bottomed cages in a temperature-controlled room (22°C) with a 12-h light/dark cycle, and fed semi purified-diets for 2 weeks. Food and distilled water were provided *ad libitum*. The synthetic diets contained (in g/kg): 200 casein, 650 starch or sucrose, 50 corn oil, 50 alphacel, 3 DL-methionine, 2 choline bitartrate, 35 AIN-76 mineral mix, 10 AIN-76A vitamin mix (ICN Biomedicals, Orsay, France).

In the first experiment, 6-week-old male and female rats, weighing 155 ± 2 g and 140 ± 3 g respectively, were randomly assigned to starch and sucrose groups (eight animals per group). Non-fasted rats were weighed, anesthetized with sodium pentobarbital (40 mg/kg body weight, intraperitoneally) and killed. Blood was collected from the abdominal aorta in heparinized tubes. Plasma obtained after low speed centrifugation (2000*g*, 15 min), was stored at −80°C for biochemical analysis. The heart was rapidly removed, washed in ice-cold saline (9 g NaCl/L), immersed in liquid N_2, and stored at −80°C before analysis.

In the second experiment, 60 weaning females, weighing approximately 60 g, were used. After anesthesia with sodium pentobarbital (40 mg/kg body weight, intraperitoneally), 40 rats were ovariectomized (20) and treated every 2 days with either placebo or 17β-estradiol (10 μg/kg/48 hr intraperitoneally). The remaining 20 rats were sham-operated. All the females were maintained on a regular chow diet for 3 weeks and then assigned to six groups as follows: Group 1: sham-operated (SH) starch-fed females, Group 2: SH sucrose-fed females, Group 3: ovariectomized (OVX) starch-fed females, Group 4: OVX sucrose-fed females, Group 5: OVX + estradiol (E2) starch-fed females, and Group 6: OVX+E2 sucrose-fed females. After 2 weeks on the experimental diet, hearts were removed as described above and stored for biochemical analysis. Principles of laboratory animal care (NIH publication No.85-23, revised 1985) were followed.

Plasma and Tissue Analysis. Plasma vitamin E was assayed by reversed-phase HPLC (HPLC apparatus; Kontron series 400; Kontron. S^t Quentin en Yvelines, France) using a hexane extract. Briefly, α-tocopherol acetate (Sigma Chemical Co., St. Louis, MO) was added to samples as an internal standard. They were then extracted twice with hexane, after ethanol precipitation of the proteins. This extract was evaporated to dryness under N_2, dissolved in ethanol-methylene chloride (65:35, v/v), and injected onto a C_{18} column (nucleosil; 250-mm long, i.d. 46 mm, 5-μm particles). Pure methanol, at a flow-rate of 2 ml/min, eluted α-tocopherol in 5 min and tocopherol acetate in 6.3 min. The compounds were detected by UV (292 nm) and quantified by internal and external calibration using daily controlled standard solutions. Triacylglycerol (Biotrol, Paris, France) was determined in plasma by enzymatic procedures. Nitrite + nitrate NOx, degradation products of nitric oxide (NO), were measured in deproteinized plasma of sucrose- and starch-fed rats. We used the Griess reaction procedure as previously described (21). Briefly, after protein precipitation of the samples and reduction of nitrate to nitrite with nitrate reductase, nitrite was quantified colorimetrically at 450 nm using a multiplate reader spectrophotometer (MR 700, Dynatech Laboratories, Guernsey, GB). Standards were made up by serial dilutions of sodium nitrite.

For lipid peroxidation studies of heart tissue, homogenates were prepared on ice in a ratio of 1 g wet tissue to 9 ml 150 mmol/L KCl using a Polytron homogenizer. Thiobarbituric acid-reactive substances (TBARS) were measured using a spectrophotometer (Uvikon 941 plus series, Kontron Instruments, St Quentin en Yvelines, France), in tissue homogenates after lipid peroxidation induced by $FeSO_4$ (2 μmol/l)-ascorbate (50 μmol/l) for 30 min in a 37°C water bath in an oxygen-free medium using a standard of 1,1,3,3-tetraethoxypropane as previously described (22). Heart homogenates, prepared on ice in a ratio of 1 g wet tissue to 9 ml 150 mmol/l KCl using a Polytron homogenizer, were used for superoxide dismutase (SOD) determination. Tissue SOD activity was determined using a Ransod kit from Randox (Randox Laboratories, Crumlin, U.K.). Briefly, the method uses xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a formazan dye. The SOD activity was measured by the degree of inhibition of the reaction, using a spectrophotometer (Uvikon 941 plus series, Kontron Instruments, St Quentin en Yvelines, France). The results were expressed in units per gram of protein. Protein content was

determined using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL) with bovine serum albumin as standard. Copper content was determined in heart tissue by a method combining dry heat and acid digestion (23). Samples were analyzed by flame atomic absorption at wavelength 324.7 nm using a Perkin-Elmer 800 atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT; 24). Heart tissue was dry-ashed at 500°C for 12 h, and the ash was dissolved in nitric acid fore analysis of copper content. National Institute of Standard and Technology Reference material (Office of Standard Reference, Gaithersburg, MD) was digested and analyzed along with samples to verify accuracy.

Statistical Analysis. Statistical analysis was performed using the "Statview" (Abacus Concepts, Inc.) software package. Results were expressed as means ± SEM Two-way analysis of variance (ANOVA), defined as $P < 0.05$, was adopted to determine the main effects (carbohydrate and gender or hormonal status) and interaction. When significant F ratios were found, the individual means were compared by PLSD-Fisher ($P < 0.05$).

Results

The sucrose-enriched diet did not affect the normal growth of the rats. The consequences of sucrose feeding on vitamin E plasma levels were markedly different between males and females. In the males, plasma α-tocopherol levels were significantly lower in the sucrose group than in the starch group, whereas in the females, plasma α-tocopherol levels were significantly higher in the sucrose group than in the starch group. Regardless of gender, sucrose feeding was accompanied by a significant increase in triglyceride (TG) plasma levels. Consequently, the α-tocopherol/TG ratio was significantly decreased in sucrose-fed males compared with starch-fed males, whereas no difference was observed in the females. Plasma NOx levels were significantly higher in the sucrose group than in the starch group, regardless of gender (Table I).

In the males, after exposure of heart tissue homogenates to iron-induced lipid peroxidation, TBARS were significantly higher in heart tissue from the sucrose group than in that from the starch group, whereas female rats fed the sucrose-based diet were partially protected. In both male and female rats, heart SOD activities were significantly decreased by sucrose feeding. However, females had higher SOD activity than males. Decreased SOD activity in heart homogenates from sucrose-fed males was accompanied by a significant decrease in heart copper level. Compared with males, females had lower heart copper levels, but copper level was not affected by the nature of the carbohydrate in the diet (Table II).

Increased body weight was observed in ovariectomized females, and estradiol treatment reversed this effect. Uteri were weighed in OVX, OVX+E2, and SH female rats to make sure ovariectomy was correctly performed. Regardless of the type of carbohydrate, uterus weights were significantly decreased in OVX compared with SH females. This loss was partially restored by estradiol supplementation. Regardless of the type of carbohydrate, OVX females exhibited increased body weight compared with SH and OVX+E2 females. After exposure of heart tissue homogenates to iron-induced lipid peroxidation, ovariectomy resulted in increased TBARS only in sucrose-fed females, whereas no difference was observed in SH groups. Estradiol supplementation reversed this effect, no difference being observed in either starch or sucrose estradiol-supplemented groups (Table III).

Discussion

The results reported here agree with those of previous studies showing that a short-term consumption of a high-sucrose diet has a pro-oxidant effect in male rats. However, here we have demonstrated first that females are protected against the pro-oxidant effect of sucrose and that modifications of hormonal status of female rats affect the response.

Previous studies in male rats have shown that a short-term consumption of a sucrose-based diet negatively affects the balance between free radical production and antioxidant defense in male rats, leading to increased lipid susceptibility to peroxidation (14, 15, 25). Higher plasma levels of TBARS and higher urinary TBARS excretion were found in the sucrose group than in the starch group, suggesting increased production of these substances from *in vivo* lipid peroxidation (14). The plasma TG-raising effect of sucrose was accompanied by lowered α-tocopherol plasma levels, and the sucrose-fed rats were less well protected against

Table I. Body Weight, Vitamin E, Plasma Triacylglycerol Concentrations, Plasma Vitamin E/TG Ratio, and Nitrite + Nitrate Levels in Male and Female Rats Consuming Starch or Sucrose Diets

	Male		Female		Two-way ANOVA*		
	Starch	Sucrose	Starch	Sucrose	Ch	Sex	Ch × Sex
Body weight (g)	262 ± 4	274 ± 6	187 ± 2	189 ± 3	NS	<0.001	NS
Vitamin E (μg/ml)	9.93 ± 0.39a	8.33 ± 0.55b	7.03 ± 0.21c	9.81 ± 0.51a	NS	NS	<0.001
Triglycerides (mmol/L)	1.60 ± 0.16	2.28 ± 0.24	1.09 ± 0.08	1.45 ± 0.08	<0.001	<0.001	NS
Vitamin E/TG (μg/mol·TG)	6.33 ± 0.54a	4.43 ± 0.47b	6.37 ± 0.63a	6.85 ± 0.46a	NS	<0.006	<0.007
NOx (μmol/l)	10.8 ± 0.6	29.8 ± 1.7	9.5 ± 1.6	26.4 ± 1.3	<0.001	NS	NS

Results are means ± SEM (eight rats per group). Ch, carbohydrate; NS, not significant. Means in the same row with different superscripts are significantly ($P < 0.05$) different [protected least significance difference (PLSD) Fisher post-ANOVA]. *P value, two-way ANOVA.

Table II. Tissue Susceptibility to Peroxidation, SOD Activity, and Cu Levels in Heart Tissue From Male and Female Rats Consuming Starch or Sucrose Diets

	Male		Female		Two-way ANOVA*		
	Starch	Sucrose	Starch	Sucrose	Ch	Sex	Ch x Sex
TBARS (nmol/g wet weight)	83.3 ± 3.8a	144.3 ± 6.6b	83.8 ± 7.6a	101.9 ± 5.9c	<0.001	<0.002	<0.002
SOD activity (U/mg protein)	39.20 ± 1.82	32.32 ± 2.72	46.49 ± 2.47	39.86 ± 1.41	<0.004	<0.002	NS
Cu (mg/g wet weight)	4.28 ± 0.17a	3.42 ± 0.25b	2.41 ± 0.25c	2.74 ± 0.18c	NS	<0.001	<0.05

Results are means ± SEM (eight rats per group). Ch, carbohydrate; NS, not significant. Means in the same row with different superscripts are significantly ($P < 0.05$) different (PLSD Fisher post-ANOVA). *P value, two-way ANOVA.

Table III. Body Weight, Uterus Weight, and Heart Susceptibility to Peroxidation in SH, OVX, or OVX + E2 Female Rats Consuming Starch or Sucrose Diets

	SH		OVX Females		OVX + E2		Two-way ANOVA*		
	Starch	Sucrose	Starch	Sucrose	Starch	Sucrose	Ch	HS	HS x Ch
Body weight (g)	214 ± 3	220 ± 3	269 ± 5	279 ± 7	213 ± 5	213 ± 3	NS	<0.001	NS
Uterus weight (g)	0.37 ± 0.03a	0.52 ± 0.03b	0.07 ± 0.01c	0.07 ± 0.01c	0.24 ± 0.03d	0.23 ± 0.03d	<0.05	<0.001	<0.005
Heart TBARS (nmol/g wet weight)	80.92 ± 3.97	86.15 ± 3.87	86.52 ± 6.99	106.67 ± 7.8	74.13 ± 3.79	73.19 ± 4.56	NS	<0.001	NS

Results are means ± SEM (10 rats per group). Ch, carbohydrate; HS, hormonal status; NS, not significant. Means in the same row with different superscripts are significantly ($P < 0.05$) different (PLSD Fisher post-ANOVA). [a]P value, two-way ANOVA.

lipid susceptibility to peroxidation, as shown by TBARS measurement in heart tissue homogenates. Also, sucrose-fed male rats display higher plasma NOx levels (15). It has been suggested that NO is involved in oxidative stress when it reacts with superoxide ($O_2^{\cdot -}$) to form peroxynitrite ($ONOO^-$; 26), but NO has also been shown to be cytoprotective through its reaction with lipid radicals and with the transition metal iron (27). The question of whether NO acts as a pro-oxidant or is increased in the sucrose group in response to oxidative stress has been addressed (15).

Here, we also found that sucrose feeding in male rats was accompanied by a significant decrease in both the plasma vitamin E level (16%) and vitamin E/TG ratio (30%). There was no difference in the vitamin E supplied by either the starch or the sucrose diet, and an influence of type of carbohydrate on vitamin E absorption is unlikely. Therefore, because vitamin E has antioxidant functions, a low vitamin E level may result from increased vitamin E utilization, suggesting the occurrence of *in vivo* oxidative stress in sucrose-fed rats. When lipoproteins are depleted of antioxidants, unsaturated fatty acids are rapidly oxidized and the lower plasma vitamin E level in sucrose-fed rats may predispose lipoproteins to subsequent oxidative stress (28). The sucrose-induced increase in TG-rich lipoprotein and increased lipoprotein susceptibility to peroxidation are factors that may combine to increase risk of cardiovascular diseases (29). Compared with starch feeding, sucrose feeding thus results in a higher occurrence of aortic atherosclerotic plaque in animal models as previously reported (30). Moreover, we found that heart tissue from male sucrose-fed rats was more susceptible to *in vitro* peroxidation than that from the starch group. The specific SOD activity was also found to be lower in male sucrose-fed rats. The decreased heart SOD activity in sucrose-fed rats was accompanied by a decrease in heart copper concentration. The interaction of dietary fructose with copper has received considerable attention (31). However, whether the decrease in SOD activity is related to copper depletion or whether the protein is damaged by oxidative stress is unclear. Nevertheless, SOD depletion may contribute to cardiac vulnerability to oxidative stress because this antioxidant enzyme plays a key role in cell protection against the detrimental effect of superoxide anion (32). Moreover, our results suggest a possible implication of oxidative stress in the cardiomyocyte dysfunction observed in sucrose-fed rats (33), and points to a possible involvement of oxidative stress, among other components of the metabolic syndrome, as an important risk factor for cardiovascular diseases.

The results reported here demonstrate for the first time that female rats are protected against the pro-oxidant effect of sucrose. It is well known that lipid metabolism is influenced by sex hormones in animals and humans (34). Hypertriacylglycerolemia resulting from sucrose feeding was lower in females than in males, suggesting that hepatic metabolism of fructose may be different in males and females, or that clearance of TG may be accelerated in female rats. Unlike other fat-soluble vitamins, vitamin E has no specific transport protein but instead is transported in plasma lipoproteins (28). The slight hypertriacylglycerolemia found in sucrose-fed female rats compared with starch-fed female rats was accompanied by higher plasma α-tocopherol concentration. The vitamin E/TG plasma ratio was not modified in sucrose-fed female rats compared with starch-fed female rats. These findings contrast with the significant decrease in vitamin E/TG plasma ratio observed when male rats were fed the sucrose diet compared with the starch diet. The

results of the first experiment emphasize the detrimental effect of sucrose on cardiovascular risk in males as shown by hyperlipidemia, increased lipoprotein, and heart susceptibility to free radical mediated injury, whereas females were protected against the pro-oxidant effect of sucrose. This prompted us to investigate whether endogenous estrogens could explain the protective effects of female gender against the pro-oxidant effect of sucrose. This was done using intact, OVX and E2+OVX females. As in previous studies, ovariectomy was accompanied by increased body weight, whereas estradiol treatment restored the normal growth of the rats (35). Uterus weights were significantly decreased by ovariectomy, confirming the effective suppression of ovarian hormonal activity. In these rats exogenous estrogen administration induced an uterotropic activity. The results clearly indicate the influence of hormonal status on heart susceptibility to lipid peroxidation. When animals were fed the sucrose diet, OVX females exhibited significantly higher TBARS values in heart tissue, and estradiol treatment reversed this effect. Increased susceptibility to lipid peroxidation in OVX females fed sucrose was not directly related to increased body weight. Hence, although this abnormal growth was also observed in OVX females fed starch, it was not accompanied by increased heart susceptibility to lipid peroxidation in those rats. The pro-oxidant effect of fructose has been discussed in relation with the lowered copper status and decreased heart Cu-Zn-SOD activity (15). Our results confirm that in male rats, decreased copper level and decreased SOD activity in heart tissue were accompanied by oxidative stress. Females were protected against the pro-oxidant effect of sucrose even though SOD activity was significantly decreased by sucrose feeding. Heart SOD activity was higher in females than in males regardless of the type of carbohydrate. However, heart copper concentration was significantly lower in female rats than in males, and copper concentration was not affected by the type of carbohydrate, suggesting an independent relation between copper metabolism and specific SOD activity in these conditions. In addition, although female rats fed a high-sucrose diet have altered heart enzyme activity, SOD activity level in this group remained similar to that of starch-fed male rats. Although the mechanisms for the pro-oxidant effect of sucrose are still unclear, the implication is that the modulation of SOD activity by sucrose feeding and the influence of gender may contribute to the pro-oxidant effect of dietary sucrose. Our experiment also confirmed previous work showing that a high sucrose diet increased plasma NOx level in male rats (15). Moreover, the effect of sucrose on plasma NOx level was also observed in female rats. Overall we conclude that female rats are protected against the pro-oxidant effect of a short-term high-sucrose diet, and that further experiments are required to determine whether the same effect is observed in female animals after longer exposure to a high sucrose diet. Protective effects of estrogens, widely described in animals and humans (36), can be mediated through genomic effects on estrogen receptors or by their antioxidant properties. It has been suggested that estrogens can suppress free radical-induced peroxidation chain reactions because of the similarity of their chemical structure with that of vitamin E, especially the presence of the hydroxyl group on the phenolic A ring (37, 38). This antioxidant capacity may be a mechanism whereby estrogens limit coronary heart diseases in women (39). Estrogens alone may protect tissues from oxidative damage resulting from sucrose feeding: treatment of ovariectomized rats with exogenous estrogens effectively restores the protection of female rats against the pro-oxidant effect of a sucrose-based diet. This hypothesis is also supported by other experiments showing the protective effects of endogenous estrogens against oxidative stress induced by copper deficiency (40). It is well known that inadequate antioxidant protection is a consequence of copper deficiency as a result of a reduction of SOD activity and that increased lipid peroxidation is a contributing factor in the pathophysiology of low copper status (41). In copper-deficient rats, feeding sucrose caused severe pathology and mortality. In contrast, the consumption of starch did not produce such pathology and the copper-deficient animals survived (42). In this latter model, female rats fed a sucrose diet are lent a greater degree of protection against the detrimental effect of copper deficiency.

It is well known that high fructose-fed rodents develop a syndrome that is the equivalent of the human metabolic syndrome (12). Previous studies have shown that female rats do not develop sucrose-induced insulin resistance (18), and that hypertension was significantly delayed in females with functional ovarian status (43). In this context, our results showing that female rats are protected against sucrose-induced oxidative stress also support the involvement of oxidative stress in this experimental model of syndrome X. Given the high prevalence of peri- and postmenopausal women in cardiac manifestations of syndrome X, it has been hypothesized that estrogen deficiency may play a major role in the pathogenesis of this conditions (19, 44). Our results showing the influence of hormonal status in female rats on heart susceptibility to oxidative damage support this postulate. Finally, our findings suggest that oxidative stress may be a primary event in the development of the cluster of abnormalities leading to syndrome X.

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