Vitamin E Improves the Free Radical Defense System Potential and Insulin Sensitivity of Rats Fed High Fructose Diets[1]

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ABSTRACT The purpose of this study was to investigate the effects of vitamin E in rats fed a high fructose diet which leads to insulin resistance, on some components of the free radical defense system and on insulin sensitivity. The rats (postweaning, 50 g) were divided into three groups: the control group (C, $n = 16$), which received a purified diet containing 60 g/100 g carbohydrates, the high fructose–fed group (FT, $n = 16$), fed a diet in which 56.8% of the carbohydrate as fructose, and a high fructose and vitamin E–fed group (FVE, $n = 16$), fed the FT diet supplemented with 3.4 g vitamin E/kg diet (vs. 0.17 g/kg in C and FT groups). The duration of the treatment was 6 wk. Insulin sensitivity was determined in half of the rats in each group using the euglycemic hyperinsulinic glucose clamp technique. The remaining rats were investigated for plasma glucose, insulin, triglyceride and fructosamine concentrations and for components of the free radical defense system. The FT group had a significantly lower insulin sensitivity than the C group. Basal glycemia was not different among the groups. In comparison with the C group, the FT group had a greater lipid peroxidation, as indicated by the higher concentrations of plasma thiobarbituric acid reactive substances (TBARS) and blood disulfide glutathione (GSSG) and the lower Cu-Zn superoxide dismutase (Cu-Zn SOD) activity. These markers approached the values of the controls after addition of vitamin E. Moreover, the FVE group had a higher insulin sensitivity than the FT group, but it remained lower than in the C group. These results show that a high fructose diet in rats leads to insulin resistance and a defect in the free radical defense system. Vitamin E supplementation improves insulin sensitivity in fructose-fed rats. J. Nutr. 127: 103–107, 1997.

KEY WORDS: *rats* • *fructose* • *insulin sensitivity* • *vitamin E* • *oxidative stress*

Varying the type of carbohydrate in the diet can influence glucose metabolism and insulin action. For example, rats consuming a high fructose diet develop insulin resistance, hypertriglyceridemia and hypertension (Thorburn et al. 1989). Several lines of evidence suggest that this diet leads to the metabolic changes observed in syndrome X, in which insulin resistance, hypertension and dyslipidemia are observed in glucose intolerant and prediabetic patients (Reaven 1988). Such metabolic modifications have been associated with a high incidence of cardiovascular disease (DeFronzo and Ferranini 1991, Reaven and Laws 1994). On the other hand, the role of free radical attack in diabetes mellitus and in the cardiovascular complications of the disease has been documented largely through the effects of free radicals on lipids and proteins (Giugliano et al. 1996, Jain et al. 1989, Oberley 1988, Ozdemirler et al. 1995). In these disease conditions, lipophilic antioxidants, such as α tocopherol, have been shown to be efficient in the protection of lipid and cell membranes against free radical attack (Esterbauher et al. 1989). Moreover, α tocopherol supplementation in patients with non-insulin-dependent diabetes mellitus (NIDDM)[3] improves insulin action (Paolisso et al. 1993). At this time, few data are available concerning some of the antioxidant system components during insulin-resistance states, without hyperglycemia, as observed in rats fed a high fructose diet. In light of such evidence, the present study investigated the free radical activity in rats fed a high fructose diet. The effects of vitamin E supplementation on insulin action was also investigated to understand the involvement of free radical attack or protection (i.e., lipid peroxidation, antioxidant enzyme activity, and trace element and glutathione concentrations) on the impaired insulin activity in rats fed a high fructose diet. These results are of interest in human nutrition, because of the increasing consumption of fructose in the western population (Economic Research Service 1989).

MATERIALS AND METHODS

Animal and experimental design. The animal care complied with the guidelines of the National Institutes of Health (NRC 1985). Male

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[3] Abbreviations used: C, control; Cu-Zn-SOD, Cu-Zn-superoxide dismutase; FT, high fructose; FVE, high fructose plus vitamin E; GIR, glucose infusion rate; GSH, reduced glutathione; GSSG, disulfide glutathione; MDA, malonedialdehyde; NIDDM, noninsulin-dependent diabetes mellitus; *R*a, rate of appearance; *R*d, rate of disappearance; Se-GSM-Px, selenium-glutathione peroxidase; TBARS, thiobarbituric reactive substances; TEP, 1,1,3,3-tetraethoxypropane.

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TABLE 1

Diet compositions of the control (C), high fructose (FT) and high fructose + vitamin E (FVE) groups

	C diet	FT diet	FVE diet
		g/100 g	
Glucose	38	15.96	15.96
Fructose	—	33.64	33.64
Wheat starch	20	8.40	8.40
Casein	23	23	23
Cellulose	6	6	6
Lard	3	3	3
Corn oil	1	1	1
Rape seed oil	1	1	1
Salt mixture[1]	7	7	7
Vitamins[2,3]	1	1	1

[1] Salt mixture expressed in g/kg: $CaHPO_4$, 30 g; KCl, 100 g; NaCl, 100 g; MgO, 10.5 g; $MgSO_4$, 50 g; $Fe2O_3$, 3 g; $FeSO_4 \cdot 7H_2O$, 5 g; trace elements 10 g/kg including: Mn, 0.8 g; CuO, 125 g; Co, 0.0009 g; Zn, 0.450 g; I, 0.0049 g.

[2] Expressed per kg of the vitamin mixture: retinol, 539 mg; cholecalciferol, 6.250 mg; thiamin, 2000 mg; riboflavin, 1500 mg; niacin, 7000 mg; pyridoxine, 1000 mg; cyanocobalamin, 5 mg; ascorbic acid, 80,000 mg; D,L-α-tocopherol acetate, 17,000 mg; menadione, 1000 mg/kg; nicotinic acid, 10,000 mg; o choline, 136,000 mg; folic acid, 500 mg; *para*-amino benzoic acid, 5000 mg; biotin, 30 mg/kg.

[3] For fructose + vitamin E diet: as fructose diet but D,L-α-tocophenyl-acetate concentration is 3.4 g/kg diet.

Wistar rats were provided by Iffa-Credo (Les Arbresles, France). The age of the rats at the initiation of the experiment was 2 wk. They were housed in stainless steel cages (4 rats/cage) and allowed free access to deionized distilled water delivered via a stainless steel watering system. Food intake was recorded every day. Weights were monitored weekly. The rats were maintained at a constant temperature (23°C), with a fixed (12-h) artificial light period. They were divided into three experimental groups: a control group (C, $n = 16$) receiving a purified diet containing 60 g/100 g carbohydrates, a high fructose–fed group (FT, $n = 16$) in which 56.8% of the carbohydrate was fructose, and a high fructose and vitamin E–fed group (FVE, $n = 16$) receiving the FT diet but supplemented with 3.4 g vitamin E/kg diet (vs. 0.17 g/kg in the C and in the FT groups). Each diet is described in **Table 1.** The rats were fed the diets for 6 wk. Eight of the rats of each group were used for the investigation of insulin sensitivity using the euglycemic hyperinsulinic glucose clamp technique. The remaining rats ($n = 8$) were investigated for metabolic and some of the free radical system components. At the end of the experiment, the rats were killed using a lethal dose of pentobarbital (Sanifi Santé Animale, Paris, France).

Preparation of the rats. The euglycemic clamp procedure was performed by the technique previously described (Rossetti et al. 1990). After intraperitoneal anesthesia (pentobarbital, 50 mg/kg, Sanifi Santé Animale), a small incision was made 0.5 cm from the cervical midline and at the level of the forelegs, and the jugular vein was exposed. After superior ligation, the vessel was catheterized with silastic tubing (0.0635 cm i.d., 0.120 cm o.d.). The same small incision permitted exposure of the carotid artery on the contralateral side for catheterization. After occlusion with an arterial clip, the vessel was catheterized with catheter PE 10 (Clay Adams, Persipany, NJ) which has been previously joined to PE 50. The segment of catheter was advanced to the carotid arch. After the vessels were catheterized and ligated securely, the catheters were tunneled subcutaneously and emerged on the dorsal side of the neck. All skin incisions were closed with a 3-0 thread; the catheters were filled with a viscous solution of polyvinylpirolidone and sealed. The catheters required no more care before the study.

Euglycemic clamp procedure. The metabolic experiment was performed 24 h after surgery (1000 h) on food-deprived conscious rats. At the beginning of the experiment, two successive (10 min) samples of blood were taken for measurement of basal glycemia. Insulin (Actrapid, Novo Nordisk, Paris, France) and glucose (1 min later) were then infused; the rate of glucose infusion was corrected manually every 5 min to maintain the desired level of glycemia. A total of 1200 μL of blood was withdrawn during the experiment for glucose measurement, performed by the glucose oxidase method on a glucose analyzer (Yellow Springs Instrument, Colombus, OH).

During insulin administration, the increase in glucose uptake by insulin-sensitive tissues was measured by the increase in the rate of disappearance (*Rd*). At a high insulin infusion rate (13,600 pmol/min), *Rd* can be determined by the glucose infusion rate (GIR) because hepatic glucose production is completely inhibited at this insulin level (Faure et al. 1994). At steady state, the rate of glucose appearance (*Ra*) is equal to *Rd* and is given by the GIR. Under these conditions, the GIR reflects the insulin sensitivity of the peripheral tissues. Because hepatic glucose production was completely inhibited at this insulin infusion rate, we did not use labeled glucose in this study (Faure et al. 1994).

Sample collections. At the end of the sixth week of dietary treatment, the rats were deprived of food overnight. Blood samples were taken via heart puncture, under anesthesia with intraperitoneal injection (50 mg/kg, pentobarbital, Sanofi Santé Animale). Laboratory tests were conducted on 10 mL of blood collected in heparinized polypropylene tubes free of trace elements (Bioblock Scientific, Illkirch, France).

Measurement of plasma triglycerides, glucose, fructosamine and insulin. Plasma triglyceride measurement was performed on an autoanalyzer (BM Hitachi, 717, Meylan, France) using a Sigma kit (339-10, Sigma Chemical, Paris, France). Glucose was measured on an autoanalyzer (BM Hitachi, 717) by the glucose oxidase method using a Boehringher Mannheim kit (166 391, Meylan, France). Fructosamine determination was also performed on an autoanalyzer (BM Hitachi, 717) using a Boehringher Mannheim kit (1 101 668). Plasma insulin was measured by RIA (kit Insulin CT, ORIS, Gif sur Yvette, France).

Determination of the non-enzymatic and enzymatic defense system components. Just after blood collection, 400 μL of whole blood was transferred to a tube containing metaphosphoric acid in water. Total glutathione (GSH + GSSG) was determined enzymatically (Akerboom et al. 1981) in the acidic protein-free supernatant. The assay of oxidized glutathione (GSSG) was performed after masking reduced glutathione (GSH) by adding 2-vinylpyridine to the deproteinized extract. After this procedure GSSG was also enzymatically determined. Plasma tocopherol was measured by HPLC (Kontron Instruments, Rotkreuz, Switzerland) using α-tocopherol acetate as the internal standard (Arnaud et al. 1991). The chromatographic separation was performed using a reverse-phase silica gel column (Alltech, Templeuvre, France) and an isocratic elution with acetonitrile. The detection was performed using spectrophotometry at 292 nm.

Trace element analysis was performed using atomic absorption spectrophotometry. For trace element measurements, plasma was removed by centrifugation (3000 × *g*, 15 min). Zinc and copper concentrations were determined using flame atomic absorption spectrophotometry (Perkin-Elmer model 460, Norwalk, CT), as described previously (Arnaud et al. 1984 and 1985). Zinc and copper concentrations were calculated using an exogenous calibration curve.

Selenium concentrations were determined by flameless atomic absorption spectroscopy after a sampling dilution procedure, with a Perkin-Elmer model 5100 fitted with a HGA 600 graphite furnace. Plasma selenium was determined by standard addition calibration curves (Neve et al. 1987). Se-glutathione peroxidase activity (Se-GSH-Px, EC 1.11.1.19) was measured by the modified method of Gunzler et al. (1974), using *tert*-butyl hydroperoxide as substrate. The results were expressed as μmol of NADPH oxidized per minute per gram of hemoglobin for erythrocyte and as international units per liter for plasma Se-GSH-Px.

Cu-Zn Superoxide dismutase activity (Cu-Zn SOD, EC 1.15.1.1) was determined by monitoring the auto-oxidation of pyrogallol according to the method of Marklund and Marklund (1974). One unit of Cu-Zn SOD activity is defined as the amount of the enzyme required to inhibit the rate of pyrogallol autoxidation by 50% and is given in μg/g hemoglobin (Hb).

TABLE 2

Plasma glucose, fructosamine, triglycerides and insulin in control (C), fructose (FT)– and fructose + vitamin E (FVE)–supplemented rats[1,2,3]

Plasma component groups	C	FT	FVE
Glucose, *mmol/L*	4.5 ± 0.3	4.9 ± 0.2	5.1 ± 0.4
Fructosamine, *μmol/L*	122.1 ± 11.3[a]	134.7 ± 12.5[b]	121.2 ± 17[a]
Triglycerides, *mmol/L*	0.45 ± 0.022[a]	2.30 ± 0.34[b]	2.37 ± 0.22[b]
Insulinemia, *pmol/L*	340 ± 100	420 ± 110	410 ± 200

[1] Values are expressed as means ± SD, $n = 8$.
[2] Samples were obtained from anesthetized rats by heart puncture after overnight food deprivation.
[3] Means in rows followed by different superscript letters are significantly different, $P < 0.05$.

Lipid peroxidation intermediates: plasma thiobarbituric acid reactive substances (TBARS). TBARS are products of the oxidative degradation of polyunsaturated fatty acids, in particular, malonedialdehyde (MDA). We used the modified method of Ohkawa et al. (1979) as described previously (Richard et al. 1992).

Statistical analysis. ANOVA was used to compare multiple group means, followed by the Newman-Keuls test (Winer et al. 1991) to determine statistical significance among the diet groups. Differences were considered significant when $P < 0.05$. All statistical analyses were performed on an IBM computer using the PCSM software package (Meylan, France). All data are expressed as means ± SD.

RESULTS

Food consumption and weight of the rats. Throughout the experiment, no differences were noted in the food intake of the three groups (data not shown). After 6 wk, the body weights of the rats were not significantly different (242 ± 11 g, C group; 249 ± 14, FT group; and 247 ± 11, FVE group).

Assessment of insulin sensitivity and metabolic status. The three groups exhibited different insulin sensitivities. In comparison with the C group which had a GIR of 172.36 ± 10.45 μmol/kg · min, a marked insulin resistance was observed in the FT group which had a GIR of 83.96 ± 8.1 μmol/kg · min) ($P < 0.001$). The FVE-supplemented group had a significantly higher GIR (118.98 ± 9.2) than the FT group, but it was significantly lower than the GIR of the C group.

In comparison with the C group, the FT group had significantly higher plasma triglycerides (**Table 2**) which were not significantly different from plasma triglycerides of the FVE group. Glycemia and insulinemia were not significantly different among the groups (Table 2). Plasma fructosamine was significantly higher in the FT group than in the other groups which did not differ from one another (Table 2).

Effects of the diet on lipid peroxidation and on the enzymatic and non-enzymatic defense system components. The FT rats had a significantly higher plasma TBARS than the C group, and the FVE group had lower TBARS than both other groups (**Table 3**). Plasma vitamin E was significantly higher in the FVE group than in both other groups, which did not differ from one another. Compared with the C group, blood GSSG was higher in the FT group and lower in the FVE group. The blood GSSG/GSH ratio was also higher in the FT rats compared with the C rats, and the FVE group had a lower blood GSSG/GSH ratio than both other groups. Red cell Se-GSH-Px was not significantly different among the groups but plasma Se-GSH-Px of the FVE group was lower than in both other groups which did not differ from one another. Moreover, in the FT group, red cell Cu-Zn-SOD was significantly lower than in the C and FVE groups. The FT and FVE groups had lower plasma zinc and selenium concentrations than the C group while plasma copper did not differ among the groups.

DISCUSSION

In this study we investigated the effect of vitamin E on insulin sensitivity in a conscious catheterized rat model of insulin resistance (high fructose–fed rats), using the euglycemic hyperinsulinic glucose clamp technique. In three other groups of rats, grown and fed in the same conditions, we investigated the antioxidant system components and the effects of vitamin E supplementation. In accord with previous studies (Tobey et al. 1982), the present results demonstrated a major impairment in whole-body insulin-stimulated glucose uptake in rats fed a high fructose diet. We also observed an impairment of the antioxidant defense systems in rats fed a high fructose diet. Thus, it is interesting to observe that vitamin E supplementation had a beneficial effect on insulin sensitivity of these rats. We did not observe any effect of vitamin E on triglyceridemia. Increases in blood triglyceride concentrations have been shown to reduce the number of insulin receptors (Bierger et al. 1984). Because the FVE group did not have lower triglyceridemia, we speculate that the beneficial effect of vitamin E on insulin sensitivity involves other mechanisms.

This study confirms the results of previous studies demonstrating that fructose feeding leads to glucose intolerance and decreases insulin sensitivity in intact animals (Thorburn et al. 1989). Several metabolic hypotheses have been advanced to explain insulin resistance in fructose-fed rats. It has been shown that chronic fructose feeding alters the activity of several enzymes regulating hepatic carbohydrate metabolism, including decreasing the activity of glucokinase and increasing glucose-6-phosphatase activity (Van Den Bergue 1986) leading to hepatic insulin resistance. In the present study, we observed a lower glucose uptake by the peripheral tissues as described previously (Thorburn et al. 1989). Glycemia was not significantly different in FT rats compared with both other groups. Triglycerides of the FT and the FVE groups were fourfold greater compared with the C group. This could be linked to a high formation of glycerol-3-phosphate leading to an increased synthesis of VLDL by liver (Beck-Nielsen et al. 1978). The metabolic defects of this animal model are different from those of the NIDDM model and closer to that designated syndrome X which includes dyslipidemia, hypertension, hyperinsulinemia and glucose intolerance, whereas glycemia following overnight food deprivation remains normal (Reaven 1988). Moreover, FT and FVE rats did not have significantly different body weights than the C rats, which is different than other experimental models of insulin resistance using genetically obese rats. This observation suggests that in the absence of hyperglycemia in rats food-deprived overnight, an enhanced oxidative stress can also be associated with insulin resistance (or its metabolic consequences), as shown by the increased plasma TBARS and the increased blood GSSG-GSH ratio.

TABLE 3

Oxidative defense system components, lipid peroxidation intermediates and trace elements in control (C), fructose (FT)– and fructose + vitamin E (FVE)–supplemented rats[1–4]

Plasma component groups	C	FT	FVE
Plasma TBARS, *μmol/L*	2.25 ± 0.28[b]	2.63 ± 0.39[c]	1.34 ± 0.41[a]
Red cell Cu-Zn-SOD, *μg/g Hb*	1.29 ± 0.17[b]	0.87 ± 0.2[a]	1.38 ± 0.10[b]
Red cell Se-GSH-Px, *U/g Hb*	156.7 ± 15.5	165 ± 24.6	140 ± 6.3
Plasma Se-GSH-Px, *U/L*	6500 ± 353[b]	6752 ± 496[b]	5697 ± 598[a]
Blood GSSG, *μmol/L*	5.25 ± 1.9[b]	7.18 ± 4.5[c]	4.35 ± 0.6[a]
Blood GSH, *μmol/L*	791.2 ± 76.1	780.5 ± 123	809 ± 86
Blood GSSG/GSH, × 10^3	6.6 ± 0.2[b]	9.2 ± 0.3[c]	5.3 ± 0.3[a]
Plasma vitamin E, *μmol/L*	33.2 ± 5.3[a]	37.7 ± 8.4[a]	58.5 ± 7.2[b]
Plasma Cu, *μmol/L*	16.5 ± 0.9	16.8 ± 1.7	16.8 ± 1.2
Plasma Zn, *μmol/L*	16.8 ± 1.7[b]	15.1 ± 1.5[a]	13.2 ± 0.8[a]
Plasma Se, *μmol/L*	3.9 ± 0.12[b]	3.33 ± 0.39[a]	2.95 ± 0.27[a]

[1] TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GSSG, disulfide glutathion; GSH, reduced glutathione.
[2] Values are expressed as means ± SD, $n = 8$.
[3] Samples were obtained from anesthetized rats by heart puncture after overnight food deprivation.
[4] Means in rows followed by different superscript letters are significantly different, $P < 0.05$.

Previous studies have shown enhanced lipid peroxidation as a consequence of experimentally induced diabetes mellitus and indicated that oxidative stress may be involved in the genesis of diabetic complications (Young et al. 1995). Furthermore, hyperglycemia per se could have a direct effect on oxidative lipid and protein modifications through the formation of glucose-derived free radicals, during the protein glycation process (Wolff et al. 1990).

Considering the role of free radical activity in insulin sensitivity, Paolisso et al. (1993) demonstrated that the administration of high doses of vitamin E during NIDDM in men is a useful tool to reduce oxidative stress and improve insulin action. In our experimental model, vitamin E supplementation also had a beneficial effect on insulin action. The effect of such a lipophilic antioxidant on insulin action originates from several mechanisms. It could be linked to a decrease in the blood GSSG-GSH ratio as previously hypothesized (Paolisso et al. 1993), leading to a better physicochemical protection of cell membranes. As previously shown (Ammon et al. 1989), cell membrane fluidity must be preserved to maintain insulin activity. Moreover, the FT rats had a higher plasma fructosamine concentration than the two other groups. In other words, enhanced plasma protein glycation occurred in high fructose–fed rats even in the absence of hyperglycemia. We observed that vitamin E supplementation also had a beneficial effect on plasma fructosamine concentration through its role as a free radical scavenger. Membrane modifications by free radical attack affect not only phospholipids but also proteins (Mikaelian et al. 1994), which could also reduce insulin activity (Ceriellio et al. 1991). Contrary to the Se-GSH-Px activity of red cells, the Cu-Zn-SOD activity of red cells was significantly lower in high fructose–fed rats. Although plasma copper was not different than in the control group, this observation could be linked to a decrease in tissue copper including liver, muscles and red cells associated with a fructose-enriched diet (Fields et al. 1984, Wapnir and Devas 1995). Because vitamin E supplementation leads to a normalization of red blood cell Cu-Zn-SOD activity, the hypothesis that the protein could be damaged by oxidative stress and/or glycation can be advanced (Hunt and Wolff 1991, Hunt et al. 1988). On the other hand, the lower red cell Cu-Zn-SOD activity in FT-fed rats could partly affect insulin sensitivity because this antioxidant enzyme has a key role in the cell protection against the deleterious effects of the superoxide anion (Nath et al. 1984). Some trace elements have an important role in free radical protection. In this study, plasma zinc and selenium concentrations were significantly lower in the FT and FVE groups. Zinc is a biological antioxidant (Bray et al. 1990), and its depletion can lead to oxidative stress (Faure et al. 1991b) and a reduced insulin sensitivity (Faure et al. 1991a). In particular, this metal is associated with the apoprotein of superoxide dismutase; thus its depletion could alter the protein as previously shown (Coudray et al. 1992). Selenium exerts its antioxidant effects through its role as a cofactor of Se-GSH-Px. In comparison with other studies (Fields et al. 1984), the diet fructose concentration was lower and the duration of the diet and treatment was shorter in the present study. Tissue selenium likely was not lowered in this study but more studies are necessary to investigate levels of tissue trace elements in our rat model.

In conclusion, the current results provide additional evidence that fructose feeding of rats leads to insulin resistance. It demonstrates for the first time that this diet has a deleterious effect on the antioxidant defense systems. Furthermore, our results provide direct evidence that vitamin E has a beneficial effect on insulin sensitivity. Further investigation is warranted to determine the roles of antioxidants and the oxygen free radicals on insulin action. Our findings are relevant in the field of human nutrition, particularly because of the increasing consumption of dietary fructose which is promoted as a healthy food for diabetic, prediabetic patients or in the general population. The increase in oxidative stress accompanying this diet could have adverse effects through an enhanced risk of cardiovascular lesions.

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