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Chronic *N*-acetylcysteine prevents fructose-induced insulin resistance and hypertension in rats

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Abstract

We examined if administration of an antioxidant compound protects against the development of insulin resistance and hypertension. Male rats were assigned randomly into four groups, and treated for 12 weeks with normal chow, normal chow plus *N*-acetylcysteine (1.5 g/day/kg), fructose (60% of diet), and fructose plus *N*-acetylcysteine. After 10 weeks, plasma triglyceride and 15-F_{2t}-isoprostane, and insulin sensitivity were measured, and after 12 weeks, pressor response to methoxamine (15–60 μg/kg min) was assessed. Relative to normal chow-fed controls, the fructose-fed rats had increased blood pressure, plasma insulin, triglyceride and 15-F_{2t}-isoprostane, and decreased insulin sensitivity; these changes were inhibited by *N*-acetylcysteine. Maximal pressor response to methoxamine was attenuated in the fructose-fed rats given *N*-acetylcysteine relative to the other three groups. Therefore, chronic treatment with *N*-acetylcysteine increases insulin sensitivity and prevents the blood pressure increase associated with fructose feeding in rats, the mechanism may involve the decrease of oxidative stress and α-adrenoceptor-mediated vasoconstriction.

Keywords: Fructose; *N*-acetylcysteine; Oxidative stress; Insulin resistance; Hypertension

1. Introduction

Hyperinsulinemia (increased plasma insulin) and insulin resistance (suppressed insulin-stimulated glucose uptake) are associated with several metabolic and cardiovascular disorders that include hypertension and diabetes mellitus (Bressler et al., 1996; DeFronzo, 1997; DeFronzo and Prato, 1996; Ginsberg, 2000). There is evidence that reactive oxygen species, such as superoxide anion and hydrogen peroxide, are involved in the pathogenesis of endothelial dysfunction (Lum and Roebuck, 2001), insulin resistance and hypertension (Evans et al., 2003). This may be via reduced production of vasodilators such as endothelial-derived nitric oxide (NO), increased inactivation of NO and/or generation of vasoconstrictors such as isoprostanes through peroxidation of arachidonic acid (Lum and Roebuck, 2001). There is also evidence that free radical scavengers, such as vitamin E (Jain and Wise, 1995), glutathione (Faure et al., 1997) and superoxide dismutase (Nakazono et al., 1991) are depressed in patients and experimental animals with hypertension and/or insulin resistance. Furthermore, treatments with antioxidants have been shown to reduce blood pressure in spontaneously hypertensive rats (Vasdev et al., 2001;2002). Moreover, treatment of diabetic animals with probucol (lipid-lowering and antioxidant) (Kaul et al., 1995) or with vitamin E (Faure et al., 1997; Lacy et al., 1998; Paolisso et al., 1993) have been shown to reduce insulin resistance. It is plausible to speculate that antioxidants have a protective role against the development of hyperinsulinemia, insulin resistance and hypertension.

The fructose-fed hypertensive rat is a model of acquired hypertension that exhibits insulin resistance,

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hyperinsulinemia and hypertriglyceridemia (Galipeau et al., 2002; Song et al., 2004, Nakazono et al., 1991). These abnormalities are associated with the human condition of metabolic syndrome X, and are important risk factors for coronary heart disease (Timar et al., 2000). The purpose of the present study was to examine if chronic treatment of fructose-fed rats with *N*-acetylcysteine, a free radical scavenger and glutathione donor, has a protective action against the progression of insulin resistance and hypertension in a rat model of metabolic syndrome.

2. Materials and methods

2.1. Animals and experimental design

Male rats (5 weeks of age, Sprague–Dawley, Charles River Laboratories, Québec, Canada) were randomly assigned into four groups as follows: normal chow (n=6), normal chow plus *N*-acetylcysteine (n=6), fructose-fed (n=8), and fructose-fed rats plus *N*-acetylcysteine (n=6). At 7 weeks of age, the rats were fed fructose (60% of diet) or normal chow, and given tap water with or without added *N*-acetylcysteine (1–2 g/l). Food consumption was measured once weekly. Fluid consumption was monitored every two days, and the amount of *N*-acetylcysteine consumed by each rat during the study was adjusted (1.5±0.2 g/day/kg body weight) according to the amount of fluid consumed. All the rats were maintained under a 12:12-h light–dark cycle, and cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. After 10 weeks of fructose and/or *N*-acetylcysteine treatment, an oral glucose tolerance test was performed to determine insulin sensitivity, and a blood sample (1.5 ml) was collected for the measurement of plasma triglyceride and 15-F_{2t}-isoprostane concentrations. After 12 weeks of treatment, intra-arterial pressure was measured and a dose–response curve to methoxamine (α-adrenoceptor agonist, 15–60 μg/kg min) was constructed in the four groups of instrumented conscious, unrestrained rats.

2.2. Oral glucose tolerance test and insulin sensitivity index

After 10 weeks of treatment, the rats were fasted for 12 h and given an oral glucose tolerance test. Each rat was given, by oral gavage, a solution containing 40% glucose (1 g/kg body wt). Blood samples were taken at 0, 10, 20, 30, 60 and 90 min after gavage for the measurements of plasma glucose and insulin via a Glucose Reagent Kit (Infinity™, Sigma) and radioimmunoassay kit (Linco Research, Inc., MO), respectively. The area under the curve for insulin was calculated using the trapezoidal method. The insulin sensitivity index was calculated from the data of plasma glucose and insulin according to the formula of Matsuda and DeFronzo (1999). It has been shown that results derived from the insulin sensitivity index correlate well with those from the euglycemic hyperinsulinemic clamp technique (Matsuda and DeFronzo, 1999).

2.3. Plasma triglyceride and 15-F_{2t}-isoprostane assay procedure

Plasma triglyceride concentration was determined with an enzymatic colorimetric kit (Thermo DMA, Inc., OH). Enzyme-linked immunoassay was used to measure free 15-F_{2t}-isoprostane concentration (pg/ml) in the plasma according to the instructions of the manufacturer (EIA Kit, Cayman Chemical, Ann Arbor, MI), but with slight modifications as follows: the plasma samples (50 μl) were added in triplicate into a 96-well plate followed by the addition of 15-F_{2t}-isoprostane acetylcholinesterase tracer and the antibody. The prepared plates were incubated overnight at room temperature. The next day, the plates were washed five times with the washing buffer, followed by the addition of Ellman's reagent. After 80 min, the plates were read at 405 nm with the operator blinded.

2.4. Blood pressure measurement

After 12 weeks of treatment, the rats were briefly anaesthetized with halothane (1.5% in air). Polyethylene cannulae (PE50) filled with heparinized saline (0.9% NaCl, 25 I.U./ml) were inserted into the left femoral vein for the administration of drugs, and left femoral artery for the measurement of blood pressure via a pressure transducer (p23DB, Gould Statham, CA, USA). Pressure signals were displayed and recorded by a BIOPAC computer System (MP 150). The rats were allowed 6 h recovery from the effects of surgery and anaesthesia before the study commenced.

A dose–response curve to methoxamine (15–60 μg/kg min), a selective α_1-adrenoceptor agonist (Bylund et al., 1994), was constructed in the four groups of conscious, unrestrained rats at dose-intervals of 3–5 min. Blood pressure was recorded prior to and at the plateau phase of response to each dose of methoxamine.

2.5. Drugs

All reagents were from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) unless otherwise stated.

2.6. Statistical analyses

All data are presented as mean±S.E.M. Data with multiple time points were analyzed by the linear model repeated measures analysis of variance (ANOVA) followed

Table 1
Baseline values of body weight, food and fluid intake, and plasma glucose, insulin, triglycerides and 15-F_{2t}-isoprostane in four groups of rats after treatment for 10 weeks with normal chow (C), normal chow plus *N*-acetylcysteine (1.5 g/l); CT, fructose (60% of diet, F), and fructose plus *N*-acetylcysteine (FT)

Parameter measured	C (*n*=6)	CT (*n*=6)	F (*n*=8)	FT (*n*=6)
Body weight (g)	622±16	570±13	617±11	567±21
Food intake (g/day)	35±2	43±5	37±3	40±2
Fluid intake (g/kg/day)	58±5	40±3*	55±4	43±4*
Plasma Glucose (mM)	8.3±0.2	9.6±0.3	10.5±0.8	9.3±0.8
Plasma Insulin (ng/ml)	1.23±0.25	0.92±0.16	2.5±0.48*	1.38±0.20#
Triglyceride (mmol/L)	1.35±0.32	1.15±0.2	5.29±0.86*	3.56±0.3*#
15-F_{2t}-isoprostane (pg/ml)	62.3±13	74.6±14.4	115.6±11.8*	52.4±14.5#

* Significantly different (P<0.05) from the control rats given normal chow (C).
# Significantly different (P<0.05) from the fructose-fed control rats (F).

by Newman–Keuls test at P<0.05. One-way ANOVA was used to analyze the data of insulin sensitivity index.

3. Results

3.1. General characteristics

There were no significant differences in bodyweight or consumption of food and fluid among the four groups at the beginning of the study (data not shown). At 10 weeks after treatment, bodyweights and fluid intake were similar between the control rats and fructose-fed rats; however, body weights and fluid intake were decreased in the groups treated chronically with *N*-acetylcysteine relative to the respective controls (Table 1). There were no significant differences in the intake of food among the groups.

3.2. Oral glucose tolerance test

After oral glucose challenge, the plasma glucose profiles were similar among all the groups (Fig. 1A). The fructose-fed rats, however, secreted more insulin than the rats given only normal chow, as indicated by the greater peak insulin response as well as the area under the curve (Fig. 1B). Chronic treatment with *N*-acetylcysteine insignificantly decreased insulin secretion in the rats fed normal chow, but significantly decreased insulin secretion in the fructose-fed rats to a similar level as that in the control rats given normal chow (Fig. 1B).

3.3. Insulin sensitivity index (ISI)

Insulin sensitivity index (calculated from the data of oral glucose tolerance test) was significantly lower in the fructose-fed rats relative to the control rats (3.8±1.0 versus 6.2±0.9, respectively). Chronic treatment of *N*-acetylcysteine significantly increased insulin sensitivity index in diabetic rats but not control rats (6.8±0.9 versus 8.9±0.8, respectively).

3.4. Plasma 15-F_{2t}-isoprostane assay

Plasma 15-F_{2t}-isoprostane and triglyceride concentrations in the fructose-fed group were markedly higher than the corresponding values in control group fed normal chow. Chronic treatment of the fructose-fed rats with *N*-acetylcysteine significantly inhibited the fructose-induced increase in

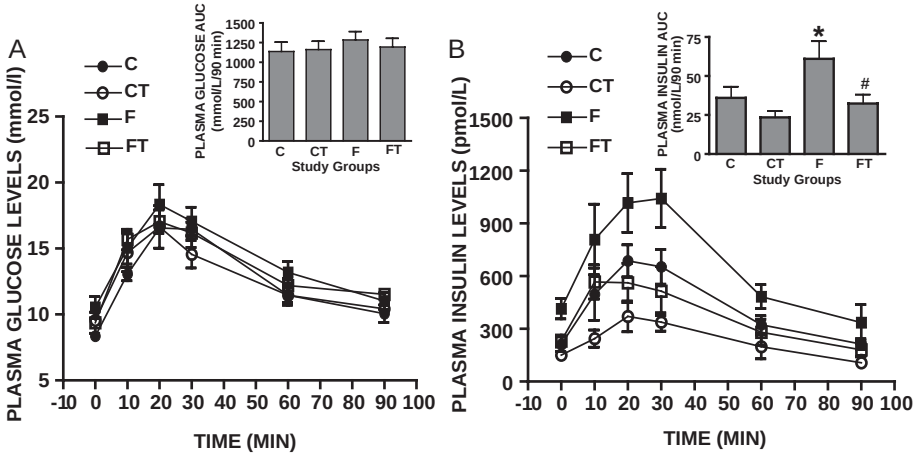

Fig. 1. Plasma glucose (A) and plasma insulin (B) versus time during an oral glucose tolerance test in groups of conscious, unstrained rats after 10 weeks of treatment with normal chow (C), normal chow plus *N*-acetylcysteine (1.5 g/day body weight; CT), fructose (60% of diet, F) and fructose plus *N*-acetylcysteine (FT). Inset, area under the curve (AUC). Values are means ± S.E.M. *Significantly different (P<0.05) from control rats given normal chow. #Significantly different from the frustose-fed rats.

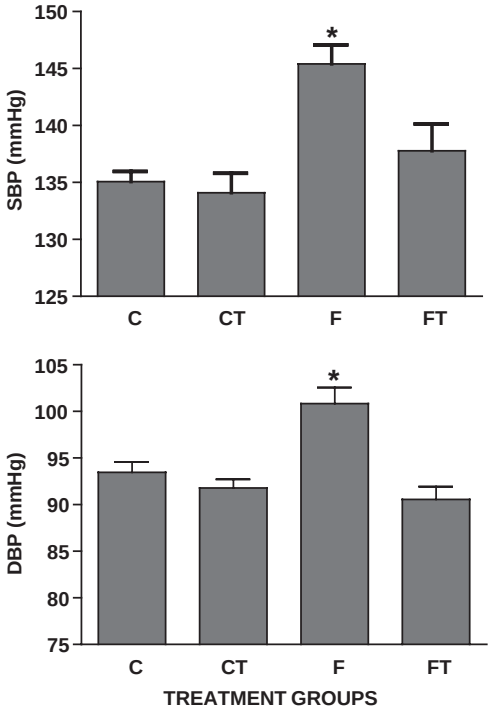

Fig. 2. Baseline intra-arterial systolic and diastolic blood pressure (SBP and DBP; mean±S.E.M.) in groups of conscious, unrestrained rats after 12 weeks of treatment with normal chow (C), normal chow plus *N*-acetylcysteine (1.5 g/day/kg body weight; CT), fructose (60% of diet, F), and fructose plus *N*-acetylcysteine (FT). *Significantly different from control rats given normal chow ($P<0.05$).

plasma triglyceride, and completely abolished the increase in plasma 15-F_{2t}-isoprostane (Table 1).

3.5. Blood pressure

At 12 weeks after the initiation of treatment, systolic and diastolic blood pressures were significantly higher in the fructose-fed group than control group given normal rat chow. Treatment with *N*-acetylcysteine prevented the increases in systolic and diastolic blood pressures caused by the fructose diet (Fig. 2).

Low doses of methoxamine (15 and 30 μg/kg/min) caused similar increases in systolic blood pressure in all groups of rats (Fig. 3). Pressor response to the high dose of methoxamine (60 μg/kg/min) was significantly decreased in the fructose-fed rats treated with *N*-acetylcysteine relative to the other three groups.

4. Discussion

The results of this study show that the feeding of a diet consisting of high fructose increases plasma insulin, triglyceride, 15-F_{2t}-isoprostane and arterial pressure, and causes the development of insulin resistance. These changes induced by a high fructose diet are in accord with the findings of other studies (Bhanot et al., 1994; Damiano et al., 2002; Huang et al., 1997; Hwang et al., 1987), and are consistent with abnormalities of syndrome X.

A possible initiating factor for the increase in arterial pressure in these rats is the generation of free radicals by a high fructose diet. A diet high in carbohydrates is associated with the generation of reactive oxygen species (Fields, 1998). Exposure of human aortic endothelial cells to high glucose increases the generation of highly reactive oxidants such as peroxynitrite and superoxide anion (Zou et al., 2002; Cosentino et al., 1997). Furthermore, elevated plasma levels of superoxide and hydrogen peroxide have been reported in patients with essential hypertension (Kumar and Das, 1993; Lacy et al., 1998) as well as the vasculature and plasma of hypertensive animals (Laursen et al., 1997; Swei et al., 1999).

In the present study, chronic treatment with the anti-oxidant *N*-acetylcysteine prevented the increase in blood pressure in the fructose-fed rats, but did not affect the blood pressure of control rats given normal chow. *N*-acetylcysteine also reduced pressor response to methoxamine in the fructose-treated rats but not normal chow-fed control rats, which indicates that the effects of NAC may involve the suppression of α_1 adrenoceptor-mediated vasoconstriction. These results are supportive of the role of free radicals in the pathogenesis of hypertension and increased vasoconstriction. There is evidence that the levels of free radical scavengers such as vitamin E, glutathione and superoxide dismutase are decreased in hypertensive patients (Kumar and Das, 1993; Sagar et al., 1992). Furthermore, a depletion of glutathione in rats via chronic administration of a glutathione synthase inhibitor has been shown to cause hypertension (Vaziri et al., 2000), and supplementation of vitamin E has been shown to decrease blood pressure both in humans and experimental animals (Boshtam et al., 2002; Pezeshk and Derick Dalhouse, 2000; Vasdev et al., 2002).

Fructose-fed rats, relative to the controls, had elevated plasma free 15-F_{2t}-isoprostane as well as triglyceride. 15-F_{2t}-isoprostane, a product of the non-enzymatic free-radical

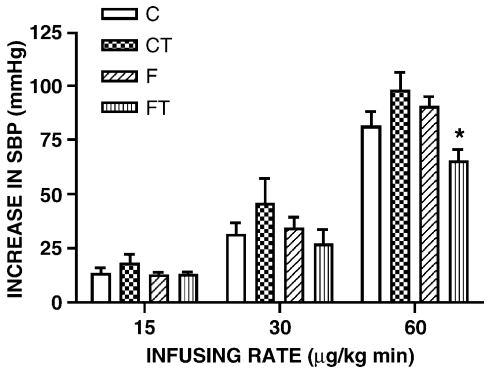

Fig. 3. Dose–response effects of methoxamine on systolic blood pressure (SBP; mean±S.E.M.) in groups of conscious, unrestrained rats after 12 weeks of treatment with normal chow (C), normal chow plus *N*-acetylcysteine (1.5 g/day/kg body weight; CT), fructose (60% of diet, F) and fructose plus *N*-acetylcysteine (FT). *Significantly different from responses to the same dose of methoxamine in the other three groups ($P<0.05$).

metabolism of arachidonic acid, is a reliable marker of lipid peroxidation and oxidative injury (Morrow and Roberts, 1997; Roberts and Morrow, 1997) as well as an efficacious vasoconstrictor (Mobert et al., 1997). Increased plasma concentration of 15-F_{2t}-isoprostane in this study therefore reflects an increase in oxidative stress, and both 15-F_{2t}-isoprostane and oxidative stress may be contributing factors in increasing the blood pressure in the fructose-fed rats. In addition to 15-F_{2t}-isoprostane, dyslipidemia and increased plasma triglycerides are also risk factors of hypertension (Zicha et al., 1999) as well as insulin resistance (Agata et al., 1998).

In the present study, *N*-acetylcysteine, in addition to preventing an increase in blood pressure in the fructose-fed rats, normalized the plasma levels of insulin and 15-F_{2t}-isoprostane, and significantly reduced plasma triglyceride and increased insulin sensitivity. *N*-acetylcysteine is a thiol-containing nucleophile that plays an important role in the scavenging of reactive oxidative radicals such as peroxynitrite (De Vries and De Flora, 1993). *N*-acetylcysteine has been shown to increase the synthesis of reduced glutathione in cultured Chinese hamster ovary cells through the promotion of cysteine uptake (Issels et al., 1988), to increase intracellular concentration of glutathione in erythrocytes, liver cells and lung cells of rats (De Flora et al., 1985), as well as to replenish reduced hepatic glutathione stores following experimental depletion in mice (Nakata et al., 1996). Chronic administration of *N*-acetylcysteine was also shown to increase the content of reduced glutathione in the aorta of the spontaneous hypertensive rats, and reduced the rats' systolic but not diastolic blood pressure (Cabassi et al., 2001). It is, however, not known if *N*-acetylcysteine prevents the development of hypertension in the fructose-induced metabolic syndrome through the scavenging of reactive oxidative radicals and prevention of insulin resistance.

In recent years, the relationship between oxidative stress and insulin resistance has attracted much attention. There is evidence that free radicals induce insulin resistance (Paolisso et al., 1994; Paolisso and Giugliano, 1996). *N*-acetylcysteine is reported to exert a protective effect on pancreatic β-cells of diabetic *db*/*db* mice (Kaneto et al., 1999), Zucker diabetic fatty rats (Tanaka et al., 1999) and alloxan-induced diabetic CD1 mice (Ho et al., 1999), and to reduce blood glucose and/or increase glucose-induced insulin secretion. In the present study, we have shown, for the first time, that chronic treatment of *N*-acetylcysteine decreased plasma insulin and improved insulin sensitivity in fructose-induced metabolic syndrome. Interestingly, the administration of metformin has been shown to reduce plasma insulin and to prevent the increase in blood pressure in rats given a high fructose diet, suggesting that hyperinsulinemia is also a risk fact of hypertension (Verma et al., 1994). Our results show that oxidative stress plays an essential role in the development of hyperinsulinemia and insulin resistance in the fructose-fed rats, and that the reduction in insulin secretion and insulin resistance may also be a contributing factor in the normalization of blood pressure.

In summary, our results show that chronic treatment of rats with a diet high in fructose causes high blood pressure, insulin resistance and oxidative stress as revealed by increased lipid peroxidation. Treatment of fructose-fed rats with *N*-acetylcysteine reduces oxidative stress and restores insulin sensitivity while inhibiting the increase in blood pressure.

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