Taurine improves insulin sensitivity in the Otsuka Long-Evans Tokushima Fatty rat, a model of spontaneous type 2 diabetes\textsuperscript{1,2}

Yutaka Nakaya, Asako Minami, Nagakatsu Harada, Sadaichi Sakamoto, Yasuharu Niwa, and Masaharu Ohnaka

ABSTRACT

Background: Taurine, a potent antioxidant, has been reported to improve streptozotocin-induced diabetes mellitus, in which the development of diabetes results from an attack by oxygen free radicals on pancreatic β cells. However, taurine also increases the excretion of cholesterol via conversion to bile acid and would be expected to improve insulin resistance.

Objective: The effects of taurine on insulin sensitivity were examined in a model rat of insulin resistance and type 2 diabetes—the Otsuka Long-Evans Tokushima Fatty (OLETF) rat.

Design: Male OLETF rats were divided into 2 groups at the age of 16 wk: a taurine-supplemented group and an unsupplemented group. As a nondiabetic control, Long-Evans-Tokushima-Otsuka rats were used. An oral-glucose-tolerance test and hyperinsulinemic euglycemic clamp were performed at the ages of 23 and 25 wk.

Results: The OLETF rats had hyperglycemia and insulin resistance and they had a greater accumulation of abdominal fat than did control rats. Abdominal fat accumulation, hyperglycemia, and insulin resistance were significantly lower in the taurine-supplemented group than in the unsupplemented group. Serum and liver concentrations of triacylglycerol and cholesterol were significantly higher in the OLETF rats than in the control rats and were significantly lower in the taurine-supplemented group than in the unsupplemented group, presumably because of the increased secretion of cholesterol into bile acid. Taurine-supplemented rats also showed higher nitric oxide secretion, evidenced by increased urinary excretion of nitrite.

Conclusion: Taurine effectively improves metabolism in OLETF rats by decreasing serum cholesterol and triacylglycerol, presumably via increased secretion of cholesterol into bile acid and decreased production of cholesterol because of increased nitric oxide production.


KEY WORDS Type 2 diabetes, taurine, Otsuka Long-Evans Tokushima Fatty rat, insulin sensitivity, visceral fat, nitric oxide

INTRODUCTION

Taurine, 2-amino ethanesulfonic acid, is a normal constituent of the human diet and is ubiquitous in tissues. Taurine is a potent antioxidant and prevents tissue injury as a result of antioxidation (1, 2). Streptozotocin-induced diabetes, a model for type 1 diabetes, is thought to result from the attack of oxygen-free radicals on β cells in the pancreas. Trachtman et al (3) reported that a dietary supplement of taurine improved streptozotocin-induced hyperglycemia in mice. However, the effect of taurine in an animal model of spontaneous type 2 diabetes has not been studied.

Most patients with diabetes have type 2 diabetes with hyperinsulinemia and insulin resistance, in which the free radical oxidation of β cells is not a cause of diabetes. In addition to antioxidant action, taurine also plays an important role in lipid metabolism, eg, such as in the enhancement of bile acid formation (4–7), which might improve lipid metabolism and insulin resistance in type 2 diabetes. Otsuka Long-Evans Tokushima Fatty (OLETF) rats are a model of type 2 diabetes with insulin resistance (8–10). These rats develop hyperphagia, obesity, hyperinsulinemia, and diabetes.

In the present study, we examined whether taurine is capable of improving insulin sensitivity and diabetic complications in OLETF rats. The results indicate that taurine is effective in improving insulin sensitivity and hyperlipidemia, presumably because of the cholesterol-lowering effect of increased bile acid production, by decreasing lipid synthesis via increased nitric oxide production, or both.

MATERIALS AND METHODS

Animals and experimental design

Male OLETF rats were fed a standard nonpurified rat diet (Oriental Yeast, Tokyo) and tap water ad libitum until the age of 16 wk, when they were randomly assigned to 2 groups of 10 rats each: those with a taurine-supplemented diet (taurine-supplemented group) and those without supplementation (unsupplemented group). Body weight and fasting blood glucose concentrations were not significantly different between the groups at the beginning of the experiment. Taurine (5%) was added to the food from 16 to 25 wk of age. A nondiabetic rat strain (Long-Evans Tokushima Otsuka) fed the same standard diet was used as the age-matched control (nondiabetic group). The standard nonpurified

\textsuperscript{1}From the Department of Nutrition, Tokushima University, School of Medicine, Tokushima, Japan.

\textsuperscript{2}Address reprint to Y Nakaya, Department of Nutrition, Tokushima University, School of Medicine, 3-18-15, Kuramoto-cho, Tokushima City, Japan 770-8503. E-mail: nakaya@nutr.med.tokushima-u.ac.jp.

Received January 13, 1999.

Accepted for publication June 1, 1999.

diet contained the following (g/100 g): protein, 23.8; fat, 5.1; starch, 54.0; fiber, 3.2; minerals; and vitamins.

After the hyperinsulinemic euglycemic clamp studies, the rats were anesthetized with sodium pentobarbital (50 mg/kg) and killed by exsanguination. Blood samples were collected from the abdominal aorta for measurement of serum concentrations of lipids and immunoreactive insulin. Abdominal fat (mesenteric, epididymal, and retroperitoneal fat) was also measured.

OGTT and euglycemic hyperinsulinemic glucose clamp

At 23 wk of age, rats underwent an oral-glucose-tolerance test (OGTT) after an overnight fast. Two grams of glucose per kilogram body weight was administered orally. Blood was drawn from a tail vein at 0, 30, 60, and 120 min for measurement of plasma glucose concentrations. Insulin-mediated whole-body glucose uptake was determined in 25-wk-old anesthetized rats by using a euglycemic insulin clamp (11). After overnight food deprivation, rats were anesthetized by intraperitoneal injection of pentobarbital, and catheters were inserted in the carotid artery and jugular vein. Rats received a 1-h infusion of insulin (60 pmol·kg$^{-1}$·min$^{-1}$). Blood glucose was measured at 5-min intervals by using the hexokinase method (Tidex; Sankyo, Tokyo) throughout the study. A glucose solution (100 g/L) was initiated at time 0; the rate was adjusted to maintain the plasma concentration of glucose at $6.1$ mmol/L. Total-body glucose uptake represents the mean glucose infusion rate (GIR) during the last 20 min.

Biochemical measurements

Serum concentrations of triacylglycerol, cholesterol, and HDL cholesterol were determined by conventional enzymatic methods (Wako, Osaka, Japan). Concentrations of cholesterol and triacylglycerol in the liver were also measured. Liver lipids were extracted by using a modification of the Folch extraction procedure (12). The plasma glucose concentration was determined by the glucose oxidase method (Toecho Super; Kyoto Daiichi Kagaku, Kyoto, Japan).

Urinary nitrite excretion rate

To determine the urinary nitrite excretion rate, 24-h urine samples from 23-wk-old rats were collected in bottles containing an antibiotic solution (1 g penicillin/L, 1 g streptomycin/L, and 0.25 g amphotericin B/L). Nitrate in urine was converted into nitrite with nitrate reductase and measured with the Griess reagent (Cyman Chemical, Ann Arbor, MI) following the manufacturer’s protocol. The nitrite measured in this manner reflected the combination of nitrite and nitrate in the original sample. Total nitrite, including nitrite converted from nitrate, is reported normalized by body weight.

Statistical analysis

Data are expressed as means ± SDs. Data were analyzed by analysis of variance plus Bonferroni multiple-comparison tests. A $P$ value <0.05 was accepted as statistically significant.

RESULTS

Body weight and abdominal fat

Body weight was significantly greater in the diabetic (OLETF) rats than in the nondiabetic rats (Figure 1). However, there were no significant differences in body weight between those with and without taurine supplementation. Food intake between 20 and 25 wk of age was not significantly different between the taurine-supplemented and unsupplemented groups (27.9 ± 3.2 compared with 28.2 ± 2.4 g/d). The amount of abdominal fat was significantly higher in the OLETF rats than in the nondiabetic rats. Total fat weight in the abdominal cavity tended to be less in the taurine-supplemented group than in the unsupplemented group, although total body weight was not significantly different between the 2 groups.

Changes in lipid metabolism

Serum concentrations of triacylglycerol and cholesterol were significantly higher in the OLETF rats than in the nondiabetic rats and significantly lower in the taurine-supplemented rats than in the unsupplemented rats (Table 1). Serum concentrations of HDL cholesterol were not significantly different among groups. The weight of the liver was significantly greater in the OLETF rats (22.1 ± 1.9 g) than in the nondiabetic rats (12.2 ± 1.2 g) and significantly lower in the taurine-supplemented group than in the
unsupplemented group (16.1 ± 1.0 g; P < 0.05). Concentrations of total fat, cholesterol, and triacylglycerol in the liver were significantly higher in the OLETF rats than in the nondiabetic rats (Table 2). The taurine-supplemented group had significantly lower concentrations of lipids in the liver than did the unsupplemented group.

**Urinalysis and blood urea nitrogen**

Urinary excretion of albumin was significantly higher in the OLETF rats than in the nondiabetic rats (Figure 2). Urinary excretion of albumin was significantly lower in the taurine-supplemented group than in the unsupplemented group. The blood concentration of urea nitrogen was significantly higher in the OLETF rats than in the nondiabetic rats [7.07 ± 0.50 mmol/L (19.8 ± 1.4 mg/dL) compared with 12.53 ± 0.57 mmol/L (35.1 ± 1.6 mg/dL); P < 0.01] and significantly lower in the taurine-supplemented group [6.53 ± 0.71 mmol/L (18.3 ± 2.0 mg/dL); P < 0.01] than in the unsupplemented group, suggesting improved renal function with taurine supplementation. Urinary excretion of nitrite and nitrate, expressed per body weight, was lower in the OLETF rats than in the nondiabetic rats. Urinary excretion of nitrite was significantly greater in the taurine-supplemented group than in the unsupplemented group.

**Glucose tolerance and in vivo glucose disposal**

Blood glucose concentrations before the OGTT were not significantly different between groups. However, blood glucose concentrations after glucose ingestion were significantly higher in the OLETF rats than in the nondiabetic rats (Figure 3). After glucose ingestion, blood glucose concentrations tended to be lower in the taurine-supplemented group than in the unsupplemented group (NS). The GIR was smaller in the OLETF rats than in the nondiabetic rats and was significantly higher in the taurine-supplemented group than in the unsupplemented group, although still lower than the value in the nondiabetic rats.

**DISCUSSION**

In the present study, the OLETF rats were obese and showed abdominal fat accumulation and impaired insulin sensitivity. Taurine supplementation improved insulin sensitivity significantly, as evidenced by lower serum lipid concentrations and less abdominal fat accumulation in the taurine-supplemented group than in the unsupplemented group. Our results suggest that the improvement in insulin sensitivity might have been due mainly to the lipid-lowering effect of taurine in the OLETF rats, in which the development of diabetes is not a free radical–related phenomenon.
insulin resistance are the primary cause of diabetes and the development of diabetes is independent of injury to β cells by free radicals. In streptozotocin-induced diabetic rats, insulin secretion is impaired, but the OLETF rats in the present study showed hyperinsulinemia at 25 wk. In the present study, taurine-supplementation significantly decreased abdominal fat accumulation and improved insulin sensitivity without a change in food intake.

Murakami et al (4, 5) reported that taurine enhanced the activity of cholesterol 7 α-monoxygenase, a rate-limiting enzyme in bile acid synthesis, and stimulated bile acid production. They also found that taurine supplementation significantly reduced concentrations of not only serum total cholesterol and triacylglycerol but of liver total cholesterol in hypercholesterolemic rats. They concluded that the reduction of serum and liver cholesterol might be due to an enhancement in the catabolism of cholesterol and the absorption of dietary cholesterol. Mizushima et al (6) reported that oral taurine supplementation attenuated increases in total cholesterol and LDL in healthy men consuming high-fat and high-cholesterol diets. In agreement with their studies, we also found that taurine supplementation tended to reduce concentrations of liver and serum cholesterol and triacylglycerol as well as abdominal fat accumulation in the OLETF rats. These results suggest that the effect of taurine on lipid and glucose metabolism was, at least in part, due to an increase in cholesterol catabolism to bile acid.

Recently, there have been many reports about the link between endothelial dysfunction and insulin resistance (13–15). These studies indicate that endothelial dysfunction might be a cause of insulin resistance. In addition, Khedara et al (16) reported that the lower nitric oxide concentration in rats fed L-N(ω)nitroarginine leads to lipidemia and that the elevations in serum triacylglycerol and cholesterol might be due to reduced fatty acid oxidation. Nitrite and nitrate are the stable degradation products of nitric oxide in biological solutions and are accurate indicators of nitric oxide production in vivo (17). The data herein show that nitrite secretion in the urine was reduced in the OLETF rats. Taurine supplementation resulted in an increased secretion of nitrite, which also might improve hyperlipidemia in OLETF rats. In agreement with our study, Kamata et al (18) reported that taurine supplementation improved endothelial-derived relaxation, in which nitric oxide is a major factor. These results suggest that improved nitric oxide production or endothelial function might also lower serum concentrations of triacylglycerol and cholesterol.

In our study, taurine decreased blood urea nitrogen concentrations and albuminuria. This might have been due to improved glucose and lipid metabolism. However, we cannot exclude the possibility of the antioxidant action of taurine because it is well known that free radical oxidation also occurs in hyperglycemia (19, 20). Trachtman et al (3) reported that the beneficial effect of taurine is related to reduced renal oxidant injury with decreased lipid peroxidation and less accumulation of AGEs within the kidney. No serious side effects were found with taurine supplementation in our study nor in the studies by Azuma et al (21) and Takahashi et al (22). These results indicate that taurine supplementation might have a beneficial effect on type 2 diabetes and its complications in a rat model of type 2 diabetes.
REFERENCES


