Troglitazone Prevents and Reverses Dyslipidemia, Insulin Secretory Defects, and Histologic Abnormalities in a Rat Model of Naturally Occurring Obese Diabetes

Dong Mei Jia, Akinari Tabaru, Hayato Nakamura, Ken-Ichiro Fukumitsu, Toshiharu Akiyama, and Makoto Otsuki

Troglitazone has been shown to improve insulin sensitivity and thereby exert hypoglycemic effects in various animal models and humans with insulin resistance and diabetes. The recently established animal model of naturally occurring obese diabetes, the Otsuka Long-Evans Tokushima fatty (OLETF) rat, has many similarities with human type 2 diabetes mellitus and is characterized by a high degree of insulin resistance. In the present study, we examined the effect of pharmacologic intervention with troglitazone on metabolic and histopathologic changes in OLETF rats. Two groups of rats received a troglitazone-rich diet (200 mg/100 g normal chow) from age 12 weeks (ie, before the onset of diabetes) or 28 weeks (ie, after the onset of diabetes) to age 70 weeks, while a third group received standard rat chow. The addition of troglitazone to the diet did not alter food intake or body weight gain. Troglitazone had no influence on visceral adipose depots, but it significantly reduced fasting glucose, insulin, cholesterol, triglyceride (TG), and free fatty acid (FFA) levels. Troglitazone reduced the insulin resistance and maintained the postglycemic insulin response at a normal level, and thus inhibited the development of insulin insensitivity and frank diabetes in OLETF rats up to 70 weeks of age. The pancreatic wet weight and insulin content were significantly higher in the treated rat groups versus the control rats. The morphologic changes observed in the control rats, such as fibrosis and structural disarrangement of islets, were minimal in the troglitazone-treated rats. Our study demonstrates that troglitazone, albeit at a dosage 10 to 15 times higher than that in humans, not only prevents but also reverses the metabolic derangement and histopathologic changes in genetically determined obese diabetes.

TYPE 2 DIABETES is associated with a decrease in insulin secretion[1,2] and a resistance to insulin action.[3,4] The impairment of insulin action in peripheral tissues (insulin resistance) has been suggested to be a prominent feature, if not the primary defect, in these patients. Treatment for type 2 diabetes consists of reducing the hyperglycemia by diet, exercise, or pharmacologic approaches such as sulfonylurea and insulin.[5] Recent reports have documented new compounds that reduce insulin resistance or potentiate insulin action in diabetic and/or obese animals.[6-10] Troglitazone belongs to the thiazolidinedione (TZD) class of medications, which have been found to improve insulin sensitivity and glucose tolerance by reducing insulin resistance via an increase in insulin-stimulated glucose disposal without stimulating B-cell insulin secretion in various animals with hyperglycemia and hyperinsulinemia[6-10] and in patients with type 2 diabetes.[11-15]

The Otsuka Long-Evans Tokushima fatty (OLETF) rat is a new inbred strain with late-onset chronic and slowly progressive hyperglycemia.[16] OLETF rats show innate polyphagia, which causes rapid body weight gain, resulting in hyperinsulinemia, hypertriglyceridemia, and hyperglycemia. At 12 to 24 weeks of age, OLETF rats are insulin-resistant but maintain normoglycemia because of a compensatory hypersecretion of insulin. Overt diabetes develops at about 20 to 28 weeks of age. After age 40 weeks, OLETF rats eventually become hypoinsulinemic and also have defects in insulin secretion.[16,17] Extreme atrophy of the pancreas and a significant reduction in the number and size of islets occur in rats older than 70 weeks of age.[16,17] Thus, the genetically obese-hyperglycemic OLETF rat has many similarities with human type 2 diabetes mellitus, and its diabetic syndrome is characterized by a high degree of insulin resistance.[16-19]

Considering the ability of troglitazone to increase insulin sensitivity and ameliorate insulin resistance in various animal models of insulin resistance and diabetes[6-10] and in type 2 diabetic patients,[11-15] the present study was undertaken to determine whether troglitazone can delay, prevent, or reverse the deterioration in the metabolic status and the histopathologic alterations in genetically obese and diabetic OLETF rats.[16-19]

MATERIALS AND METHODS

Animals and Diet

OLETF rats and their control counterpart Long-Evans Tokushima Otsuka (LETO) male rats at 5 weeks of age were kindly supplied by the Tokushima Research Institute, Otsuka Pharmaceutical (Tokushima, Japan). They were maintained in a temperature (23° ± 2°C)- and humidity (55% ± 5%)-controlled room with a 12-hour light-dark cycle with lights on at 7 AM. The animals were provided ad libitum standard rat chow consisting of (as a percent of calories) 61% carbohydrate, 26% protein, and 13% fat (Oriental Yeast, Tokyo, Japan) and tap water. They were maintained according to the ethical guidelines of our institution, and the experimental protocol was approved by the animal welfare committee.

Administration of Troglitazone

Standard rat chow was pulverized to a fine powder, and troglitazone (a generous gift from Sankyo, Tokyo, Japan) was added and thoroughly mixed to a final concentration of 200 mg/100 g food. This concentration was chosen based on previous studies that elicited an insulin-sensitizing response in diabetic mice and rats.[7,20,21] The drug-chow powder mixture was reconstituted into pellets with a normal appearance. Chow for

From the Third Department of Internal Medicine, University of Occupational and Environmental Health, Japan, School of Medicine, Kitakyushu, Japan.

Submitted October 15, 1999; accepted February 22, 2000.

Supported in part by Grants-in-Aid for Scientific Research (10470144) from the Ministry of Education, Science, Sports and Culture, Japan, and the Japanese Ministry of Health and Welfare (Intractable Diseases of the Pancreas).

Address reprint requests to Makoto Otsuki, MD, PhD, Third Department of Internal Medicine, University of Occupational and Environmental Health, Japan, School of Medicine, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan.

0026-0495/00/4909-0001$10.00/0

doi:10.1053/meta.2000.8599

control rats was prepared in a similar fashion but without the addition of troglitazone.

Experimental Protocol

The animals were fed standard rat chow until the start of the experiment. Male OLETF (O) and LETO (L) rats were randomly divided into 3 groups at 12 weeks of age (the start of the experiments). The first group was maintained on the troglitazone-rich chow from 12 weeks of age, ie, before the onset of diabetes, until the end of the study at 72 weeks of age (O-Tro12 and L-Tro12). The second group received standard chow without troglitazone until 28 weeks of age, and thereafter, ie, after the onset of diabetes, they received troglitazone-rich chow until the end of the study (O-Tro28 and L-Tro28). The control group received standard rat chow without troglitazone (O-Cont and L-Cont).

All groups were allowed free access to food and water throughout the study. The animals were weighed on a weekly basis, and food intake was determined every 2 weeks over a 48-hour period by weighing the full food cups and then weighing the food cups again 48 hours later, correcting for spillage. The mean food intake was estimated as the amount of food consumed per cage.

Intravenous Glucose Tolerance Test

At 12, 16, 20, 28, 32, 36, 44, 52, 60, and 70 weeks of age, an intravenous glucose tolerance test (IVGTT) was performed after an overnight fast. The animals were weighed before the experiments, and anesthesia was induced using sodium pentobarbital (50 mg/kg body weight intraperitoneally). A bolus dose of glucose 200 mg/kg body weight was injected into the right jugular vein immediately after blood sampling for measurement of serum insulin, glucose, cholesterol, triglyceride (TG), free fatty acid (FFA), aspartate aminotransferase and alanine aminotransferase. Blood samples were collected again from the left jugular vein at 5, 10, 30, and 60 minutes for measurement of serum glucose and insulin. Changes in insulin and glucose (Δ) were determined by subtracting the prestimulus serum level from the value obtained at 5 minutes after the bolus glucose injection for each animal. The insulinogenic index was calculated as Δinsulin/Δglucose.

Pancreas Weight, Abdominal Fat Weight, and Pancreatic Insulin Content

Two weeks after the last IVGTT (72 weeks of age) and after an overnight fast, the rats were treated with sodium pentobarbital and the abdomen was quickly opened to remove the pancreas. Retroperitoneal, mesenteric, and epididymal white adipose depots were dissected and weighed. The pancreas was excised, cleared of lymph nodes and fat, and weighed. Portions of each pancreatic tissue with similar anatomic orientation were used for histologic examination and biochemical determinations.

A portion of the pancreatic tissue was homogenized in saline using a motor-driven, Teflon-coated (E.I. du Pont de Nemours, Wilmington, DE) glass homogenizer at 3,000 rpm (8 passes). The homogenates were filtered through 3 layers of gauze and then sonicated for 1 minute. The aqueous phase obtained after 15 minutes of standing was used for protein and DNA assay. Insulin was extracted by a modified method of Davoren.[22]

Histologic Examination

A portion of the pancreatic tissue was fixed overnight in 10% formaldehyde solution for both hematoxylin-eosin and Azan staining and light-microscopic examinations. All histologic samples were examined in a single-blind fashion by the pathologist without awareness of the treatment.

Assays

Serum glucose was determined by the glucose-oxidase method using a glucose kit (Glucose-E reagent; International Reagents, Kobe, Japan).[23] Insulin concentrations in the serum and pancreatic homogenates were measured by radioimmunoassay using the double-antibody method[24] with a commercially available radioimmunoassay kit (ShionoRIA; Shionogi Pharmaceutical, Osaka, Japan) using crystalline rat insulin as a reference standard. Serum TG, FFA, and total cholesterol concentrations were measured by an enzymatic colorimetric method using commercially available kits (Wako Pure Chemical, Tokyo, Japan). The DNA concentration in pancreatic homogenates was determined by the method of Labarca and Paigen[25] using the fluorescent dye H-33258 (Hoechst, Frankfurt, Germany) and calf thymus DNA (type I; Sigma Chemical, St Louis, MO) as standards, respectively. All samples were analyzed at least in duplicate.

Statistical Analysis

Values are expressed as the mean ± SEM. Differences between groups were tested for statistical significance using ANOVA followed by Tukey's test. A P value less than .05 denoted a statistically significant difference.

RESULTS

Food Consumption and Body Weight

OLETF rats at 6 weeks of age consumed more food than LETO rats (26.1 ± 0.8 *v* 18.9 ± 0.5 g/d per rat, $P < .001$), and the mean food intake slightly increased to 30.3 ± 0.3 g/d per rat at 30 weeks of age ($P < .01$ *v* 6 weeks of age) and thereafter remained at nearly the same level until the end of the study (Fig 1). Food intake in the control LETO rats increased with age, reaching 22.7 ± 0.4 g/d per rat at 12 weeks of age ($P < .001$ *v* 6 weeks of age), and it remained at nearly the same level until the end of the study (21.7 ± 0.5 g/d per rat at 70 weeks of age). The addition of troglitazone to the diet did not influence food intake in both strains of rats. Thus, the daily dosage of troglitazone in OLETF rats was approximately 53 to 60 mg per rat, whereas that in LETO rats was 45 mg per rat.

OLETF rats at 6 weeks of age were already significantly heavier than LETO rats at the corresponding age (229 ± 5 *v* 190 ± 2 g, $P < .001$). The body weight of both strains of rats increased progressively with age, reaching a plateau at 44 to 52 weeks of age (Fig 1). Troglitazone administration did not notably influence the body weight gain in both OLETF and LETO rats.

Fasting Serum TG, FFA, and Cholesterol

At the age of 6 weeks, fasting serum TG levels were already higher in OLETF versus LETO rats (0.97 ± 0.06 *v* 0.42 ± 0.03 mmol/L, $P < .001$) and increased progressively with age (Fig 2 and Table 1). Supplementing the diet with troglitazone from 12 weeks of age (O-Tro12) significantly decreased serum TG from 1.27 ± 0.05 mmol/L at age 12 weeks to 1.05 ± 0.07 mmol/L at age 16 weeks ($P < .01$ *v* 12 weeks), and it further decreased to 0.78 ± 0.08 mmol/L at 28 weeks of age. Thereafter, serum TG increased, but it was significantly lower than the level in control OLETF rats at the corresponding age. The addition of troglitazone from 28 weeks of age (O-Tro28) also significantly decreased serum TG from 1.66 ± 0.05 mmol/L at age 28 weeks to 1.27 ± 0.08 mmol/L at age 32 weeks ($P < .001$ *v* 28 weeks),

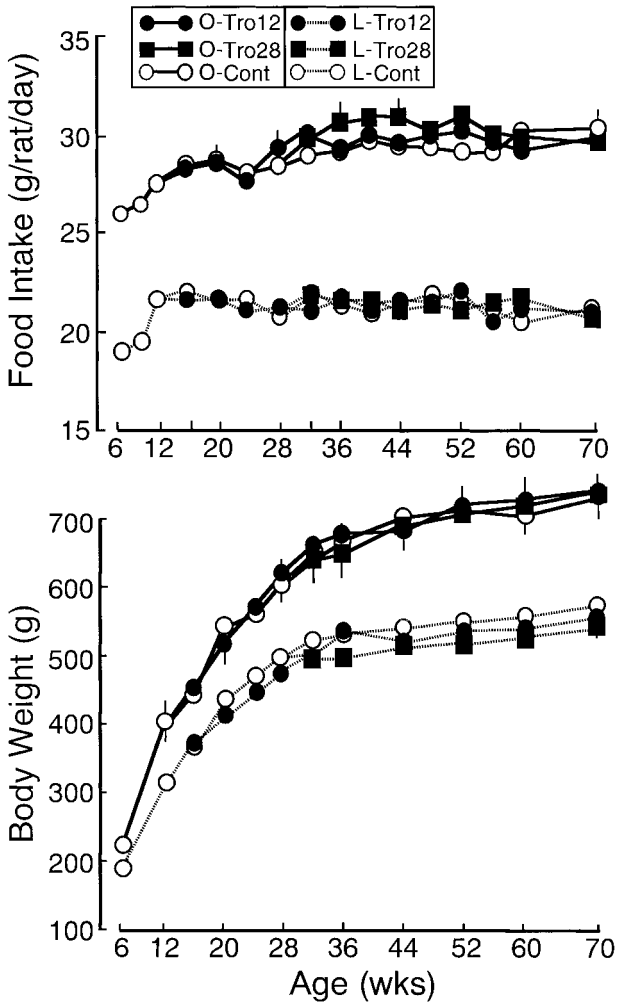

Fig 1. Serial changes in daily food intake and body weight in untreated control and troglitazone-treated OLETF and LETO rats. Results are the mean ± SEM of 6-10 rats. Because there was no difference in food intake and weight gain between the untreated control and troglitazone-treated groups (Tro12 and Tro28) before the start of troglitazone administration, the data from both groups were pooled to represent the control to simplify the Figure.

but it gradually increased after 40 weeks of age. In contrast to OLETF rats, serum TG in LETO rats remained constant until the end of the study. Troglitazone administration had no influence on serum TG levels in LETO rats, except at 70 weeks of age (Fig 2 and Table 1).

The fasting serum FFA concentration in OLETF rats at 6 weeks of age was nearly the same as that in LETO rats, but it increased progressively with age in OLETF rats (Fig 2 and Table 1). Supplementing the diet with troglitazone from 12 or 28 weeks of age in OLETF rats decreased serum FFA to less than the initial value obtained at 12 weeks of age. On the other hand, serum FFA levels in troglitazone-treated LETO rats fluctuated. Serum FFA concentrations in L-Tro12 rats were significantly high at 16 weeks of age, whereas they were significantly low at 24, 28, 44, and 70 weeks of age, compared with the values in untreated LETO rats at the corresponding age.

Fasting serum cholesterol concentrations in OLETF rats at 6 weeks of age (2.58 ± 0.07 mmol/L) were nearly the same as those in LETO rats (2.43 ± 0.07 mmol/L), and they decreased to 2.19 ± 0.10 and 1.98 ± 0.05 mmol/L, respectively, at 12 weeks of age ($P < .01$). Serum cholesterol in control OLETF rats gradually increased with age, and it was significantly higher after 28 weeks of age compared with 12 weeks (Fig 2). Troglitazone administration greatly suppressed the age-dependent increase in serum cholesterol irrespective of whether it was used from age 12 weeks (O-Tro12) or 28 weeks (O-Tro28). Supplementing the diet with troglitazone in LETO rats from age 12 weeks (L-Tro12) or 28 weeks (L-Tro28) did not cause any definite tendency in serum cholesterol values, although they were significantly lower than the levels in control rats (L-Cont) at 70 weeks of age (Fig 1 and Table 1).

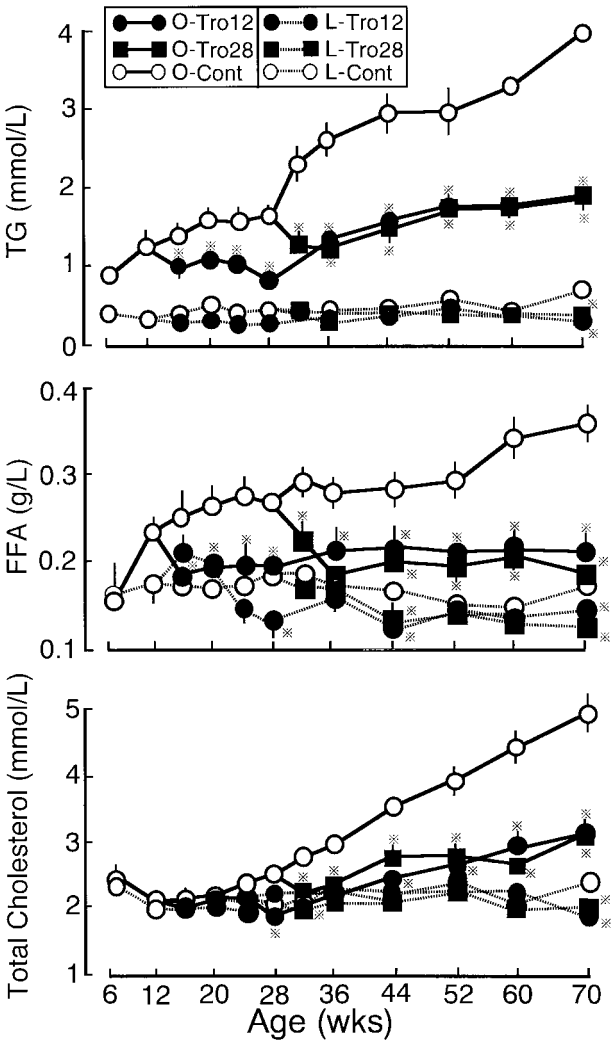

Fig 2. Serial changes in fasting serum TG, FFA, and cholesterol in untreated control and troglitazone-treated OLETF and LETO rats. Results are the mean ± SEM of 6-10 rats. Because there was no difference in serum TG, FFA, and cholesterol between the untreated control and troglitazone-treated groups (Tro12 and Tro28) before the start of troglitazone administration, the data from both groups were pooled to represent the control to simplify the Figure. *Significantly different *v* respective control at the corresponding age (*v* O-Cont or L-Cont).

Table 1. Effect of Troglitazone Treatment From Age 12 Weeks or 28 Weeks on Fasting Serum Glucose, Insulin, and Lipids and the Insulinogenic Index After an IVGTT in OLETF and LETO Rats

Fasting Serum Concentration at 70 Weeks	LETO			OLETF		
	L-Cont	L-Tro12	L-Tro28	O-Cont	O-Tro12	O-Tro28
Glucose (mmol/L)	7.44 ± 0.39	6.66 ± 0.17	6.22 ± 0.08*	13.5 ± 4.7*	9.45 ± 0.26*†	9.36 ± 0.43*†
Insulin (pmol/L)	330 ± 30	255 ± 30	255 ± 30	270 ± 15*	375 ± 30*	345 ± 15
Cholesterol (mmol/L)	2.49 ± 0.05	1.98 ± 0.05*	2.07 ± 0.04*	4.97 ± 0.24*	3.16 ± 0.13*†	3.16 ± 0.16*†
TG (mmol/L)	0.71 ± 0.04	0.33 ± 0.01*	0.37 ± 0.04*	4.07 ± 0.07*	1.88 ± 0.10*†	1.86 ± 0.04*†
FFA (g/L)	0.18 ± 0.01	0.15 ± 0.02	0.13 ± 0.02*	0.37 ± 0.02*	0.20 ± 0.01*†	0.20 ± 0.01*†
Insulinogenic index ($\times 10^{-9}$)	82.5 ± 3.7	100.3 ± 3.5*	87.3 ± 9.7	16.0 ± 2.6*	98.5 ± 9.7†	84.2 ± 7.1†

NOTE. Values are the mean ± SEM of 6-10 rats. The insulinogenic index was calculated as Δinsulin/Δglucose at 5 minutes after the bolus injection of glucose 0.2 g/kg body weight.

*Significant difference *v* L-Cont.

†Significant difference *v* O-Cont.

In OLETF rats, fasting serum TG levels were significantly higher than those in LETO rats at 6 weeks of age. Fasting FFA and cholesterol in OLETF rats, which were nearly the same as the values in LETO rats at age 6 weeks, increased progressively with age, whereas in LETO rats they remained nearly constant until the end of the study (Fig 2). The difference between OLETF and LETO rats in serum FFA levels became significant at 12 weeks of age (0.24 ± 0.01 *v* 0.18 ± 0.01 g/L, $P < .01$), and for cholesterol, the difference became significant at 28 weeks of age (2.51 ± 0.06 *v* 2.21 ± 0.12 mmol/L, $P < .01$).

Serum Insulin and Glucose Response to IVGTT

At age 6 weeks, fasting serum glucose was already significantly higher in OLETF versus LETO rats (6.7 ± 0.1 *v* 5.7 ± 0.2 mmol/L, $P < .01$), and it increased progressively with age to 13.5 ± 0.4 mmol/L at age 70 weeks (Fig 3). After an

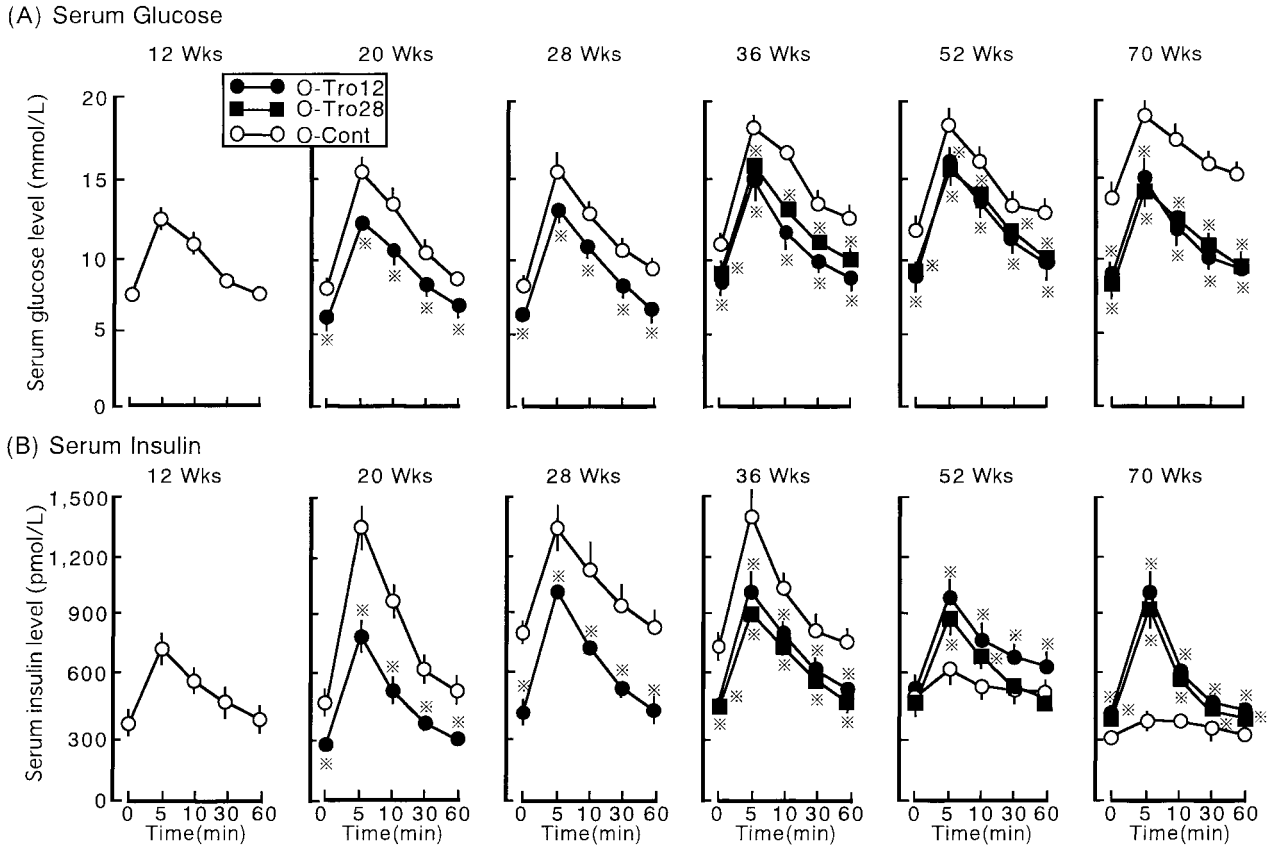

Fig 3. Serial changes in serum glucose and insulin responses to an IVGTT (0.2 g/kg body weight) in OLETF rats. Results are the mean ± SEM of 6-10 rats. Because there was no difference in serum glucose and insulin between the untreated control and troglitazone-treated groups (Tro12 and Tro28) before the start of troglitazone administration, the data from both groups were pooled to represent the control to simplify the Figure. *Significantly different *v* untreated control (O-Cont) at the corresponding time point.

intravenous glucose load, serum glucose increased, reaching a peak at 5 minutes, and then decreased to the baseline level at 60 minutes. In control OLETF rats, the glycemic response to an IVGTT gradually increased with age, reaching peak values at age 36 weeks (18.6 ± 0.3 mmol/L), which remained constant until age 70 weeks (peak, 19.2 ± 0.5 mmol/L). Supplementing the diet with troglitazone from 12 weeks of age decreased fasting serum glucose from 7.4 ± 0.2 mmol/L at 12 weeks of age to 6.4 ± 0.1 mmol/L at 24 weeks of age ($P < .01$), but thereafter it gradually increased with age to 9.5 ± 0.2 mmol/L at age 70 weeks. Troglitazone administration from 28 weeks of age in OLETF rats almost completely prevented the age-dependent increase in serum glucose at fasting (O-Cont at age 28 weeks *v* O-Tro28 at age 70 weeks, 8.8 ± 0.2 *v* 9.3 ± 0.4 mmol/L, NS) and after an IVGTT (peak O-Cont at age 28 weeks *v* O-Tro28 at age 70 weeks, 15.7 ± 2.2 *v* 14.8 ± 0.4 mmol/L, NS) (Fig 3).

Basal serum insulin concentrations were significantly higher in OLETF rats versus LETO rats at 6 weeks of age (168.0 ± 7.5 *v* 106.5 ± 13.5 pmol/L, $P < .01$) and increased progressively with age to 891.0 ± 51.0 pmol/L at age 32 weeks. However, basal serum insulin markedly decreased to 262.5 ± 21.0 pmol/L at 70 weeks of age. At 20 weeks of age, serum insulin was significantly higher at basal and showed an exaggerated response to an IVGTT in OLETF rats compared with 12-week-old O-Cont rats or L-Cont rats at the corresponding age (Fig 3). The basal insulin concentration was significantly higher but the peak insulin response was nearly the same in 28-week-old O-Cont rats versus 20-week-old O-Cont rats. At 52 weeks of age, despite a great increase in serum glucose from 12.8 ± 0.4 mmol/L to 18.9 ± 0.3 mmol/L, insulin showed only a small increase from 468.0 ± 37.5 pmol/L at baseline to a peak of 652.5 ± 27.0 pmol/L at 5 minutes after an IVGTT. At 70 weeks of age, the basal insulin concentration further decreased and showed only a small postglycemic increase (basal *v* peak, 262.5 ± 21.0 *v* 349.5 ± 33.0 pmol/L, $P < .05$). Troglitazone treatment prevented the increase of (O-Tro12) or markedly decreased (O-Tro28) the fasting serum insulin concentration (O-Cont at age 28 weeks *v* O-Tro28 at 32 weeks, 783.0 ± 76.5 *v* 453.0 ± 64.5 pmol/L, $P < .001$) until the end of the study. Moreover, the insulin secretory response to an IVGTT at 70 weeks of age remained nearly the same as the response at 12 weeks of age. Thus, the insulinogenic indices of troglitazone-treated OLETF rats (O-Tro12 and O-Tro28) were greatly improved versus the untreated control OLETF rats (Table 1).

In LETO rats, the fasting serum glucose increased slightly but significantly with age from 5.7 ± 0.3 mmol/L at age 6 weeks to a peak level of 7.4 ± 0.4 mmol/L at 70 weeks ($P < .01$). The glycemic response to an IVGTT also increased with age, reaching peak values at 70 weeks (Fig 4). On the other hand, fasting and postglycemic serum insulin concentrations showed no significant changes up to 70 weeks of age. Supplementing the diet with troglitazone in LETO rats from age 12 weeks (L-Tro12) or 28 weeks (L-Tro28) almost completely prevented the age-dependent increases in serum glucose both at fasting and after an IVGTT, and it also tended to decrease serum insulin basally and after an IVGTT (Fig 4 and Table 1).

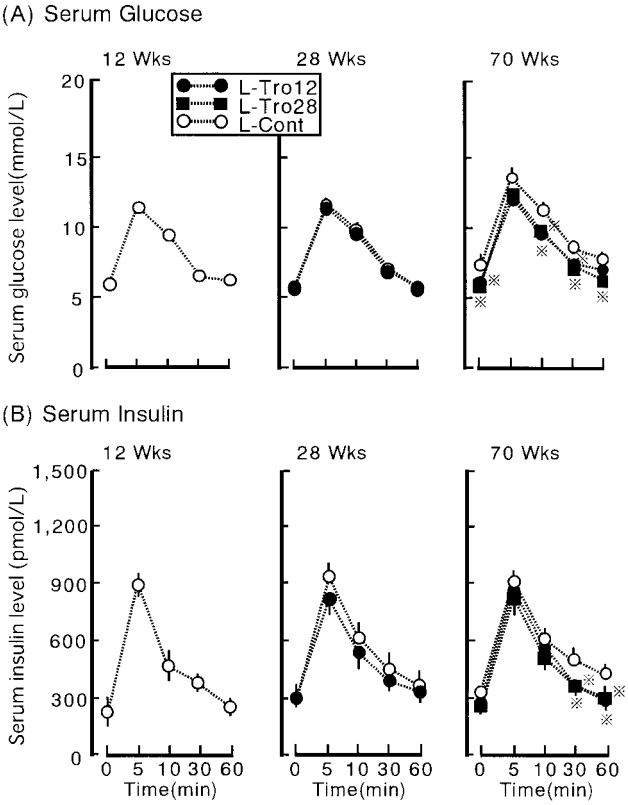

Fig 4. Serial changes in serum glucose and insulin responses to an IVGTT in LETO rats. Results are the mean ± SEM of 6-10 rats. Because there was no difference in serum glucose and insulin between the untreated control and troglitazone-treated groups (Tro12 and Tro28) before the start of troglitazone administration, the data from both groups were pooled to represent the control to simplify the Figure. *Significantly different *v* untreated control (L-Cont) at the corresponding time point.

Pancreas Weight, Abdominal Fat Weight, and Pancreatic Insulin Content

At 72 weeks of age, the pancreatic wet weight was significantly lower in control OLETF rats versus LETO rats (O-Cont *v* L-Cont, 899 ± 39 *v* 1,248 ± 39.0 mg per rat, $P < .01$). Treatment with troglitazone greatly increased the pancreatic wet weight in both rat strains. Pancreatic wet weight in groups O-Tro12 (1,216 ± 71.0 mg per rat) and O-Tro28 (1,192 ± 58.0 mg per rat) was similar to the value in L-Cont rats. Total adipose depots (mesenteric, retroperitoneal, and epididymal) were 3 times as large in OLETF rats versus LETO rats (Table 2). Supplementing the diet with troglitazone did not notably influence the total adipose depots in both strains.

Pancreatic insulin content was significantly lower in untreated control OLETF rats versus control LETO rats (Table 1). Troglitazone treatment doubled the insulin content in OLETF rats (O-Tro12 and O-Tro28) irrespective of its expression as the total amount of insulin or the amount relative to the DNA content (Table 2). Troglitazone also increased pancreatic insulin content in LETO rats, although it was significantly lower versus untreated LETO rats when expressed as the amount relative to the DNA content.

Table 2. Effect of Troglitazone Treatment From Age 12 Weeks or 28 Weeks to Age 72 Weeks on Body Weight, Adipose Tissue Weight, and Pancreatic Insulin Content in LETO and OLETF Rats

Parameter	LETO			OLETF		
	L-Cont	L-Tro12	L-Tro28	O-Cont	O-Tro12	O-Tro28
Body weight (g)	553 ± 10	525 ± 11	535 ± 14	696 ± 13*	705 ± 22*	713 ± 19*
Total adipose tissue weight						
g/rat	29.3 ± 2.1	26.5 ± 1.9	24.5 ± 1.4	97.3 ± 6.7*	91.2 ± 9.2*	92.2 ± 6.9*
mg/g body weight	53.0 ± 3.8	50.5 ± 3.6	45.8 ± 2.6	139.8 ± 7.6*	129.4 ± 13.0*	129.3 ± 9.7*
Pancreatic insulin content at age 72 weeks						
nmol/pancreas	19.0 ± 0.9	22.1 ± 1.5	25.7 ± 1.9*	10.3 ± 1.0*	26.7 ± 1.5*†	23.6 ± 0.9*†
nmol/mg DNA	3.33 ± 0.16	2.24 ± 0.22*	2.18 ± 0.25*	1.52 ± 0.25*	3.24 ± 0.10*†	3.06 ± 0.25*†

NOTE. Values are the mean ± SEM of 6-10 rats.
*Significant difference *v* L-Cont.
†Significant difference *v* O-Cont.

Histopathologic Changes

Representative photomicrographs of randomly selected sections of the pancreas at 72 weeks of age for different treatment groups are shown in Fig 5 using the same magnification. The pancreas of untreated OLETF rats was atrophic, and prominent fibrosis, fatty replacement, and tubular complexes were observed in the degenerated exocrine pancreas. The number of islets was decreased, and the contour of the islets was irregular with structural disarrangement and fibrosis. Moreover, the islets were separated into small sections (clusters) by fibrosis (Fig

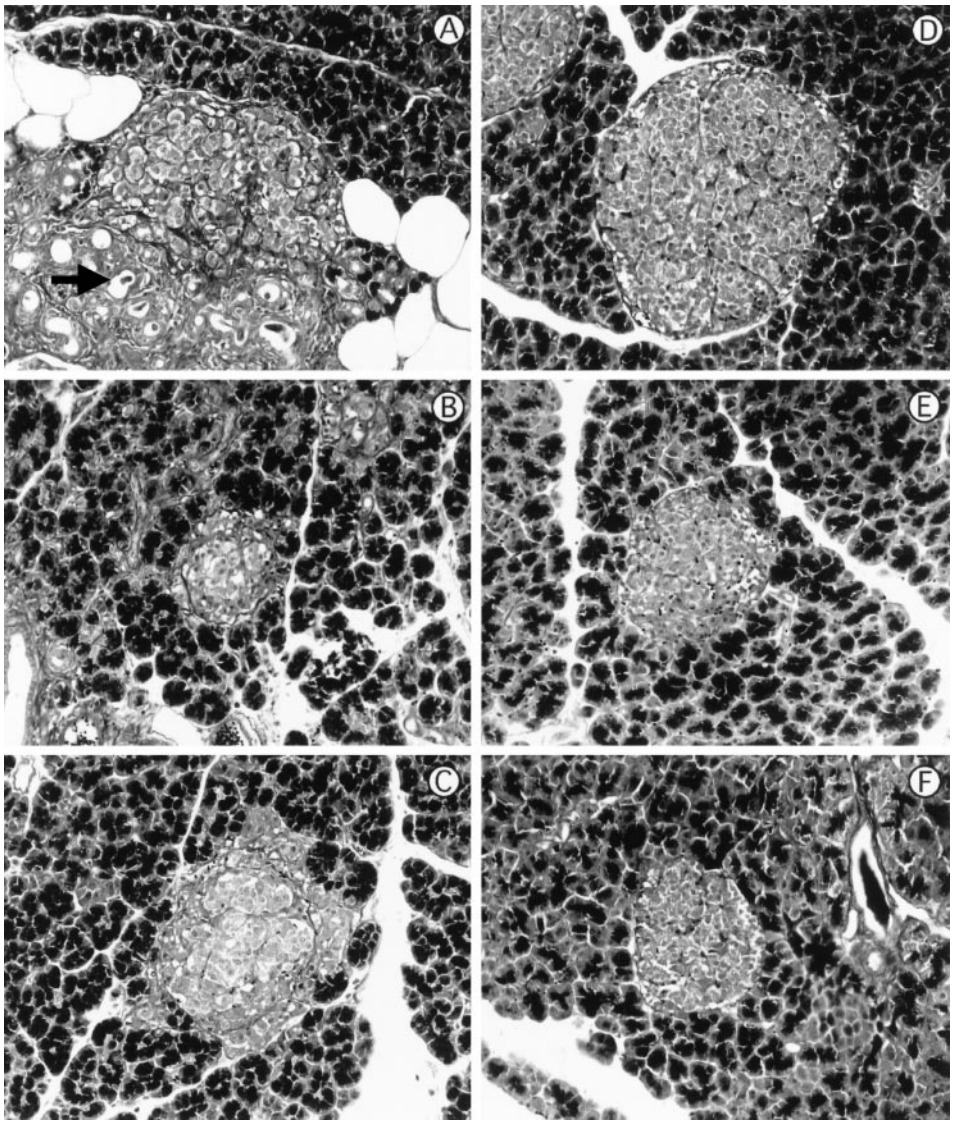

Fig 5. Representative photomicrographs of islets from untreated control and troglitazone-treated groups (Tro12 and Tro28) at 72 weeks of age. Note the differences in islet size and degree of connective tissue proliferation. (A) Pancreas of a representative O-Cont rat showing fatty replacement of the exocrine pancreas. The number of islets was decreased and the contour of the islets was irregular, with structural disarrangement and fibrosis. Note also that the islet was separated into small sections (cluster) by fibrosis and that tubular complexes (arrow) developed in the damaged exocrine pancreas. Islets of O-Tro12 (B) and O-Tro28 (C) rats were smaller and fewer compared with L-Cont (D). Note the minimal connective tissue proliferation and the lack of fatty changes. Islets from L-Tro12 (E) and L-Tro28 (F) rats tended to be smaller and fewer *v* untreated LETO rats (D). (Azan stain; original magnification ×50).

5A). On the other hand, the number and size of the islets in groups O-Tro12 (Fig 5B) and O-Tro28 (Fig 5C) appeared to be fewer and smaller compared with untreated LETO rats (Fig 5D), with minimal connective tissue proliferation. Fatty changes and tubular complexes were not observed. The histologic changes in islets remained minimal. The islets of troglitazone-treated LETO rats (L-Tro12, Fig 5E; L-Tro28, Fig 5F) tended to be smaller and fewer in comparison to the untreated LETO rats.

DISCUSSION

The results of the present study demonstrate that troglitazone administered long-term from before the onset of diabetes (12 weeks of age) or after the onset of diabetes (28 weeks of age) in the type 2 diabetic model, OLETF rats, as a 0.2% food admixture not only prevents but also dramatically improves hyperglycemia, hyperinsulinemia, hyperlipidemia, and severe histopathologic changes of pancreatic islets to near-normal levels up to 70 weeks of age, without reducing food intake or body weight gain.

OLETF rats exhibit a progressive impairment of insulin secretion that resembles human type 2 diabetes. It has been reported that body weight, serum glucose and insulin at fasting and after an oral glucose load, and insulin sensitivity at the age of 3.5 weeks are not significantly different in OLETF rats versus LETO rats.[26] Obesity with marked accumulation of intraabdominal visceral fat appears at about 5 weeks of age and develops throughout life,[18] possibly due to a satiety deficit induced by a lack of cholecystokinin A (CCK-A) receptor,[27] which is followed by insulin resistance.[16-19] In the present study, fasting serum glucose and insulin were already significantly high at 6 weeks of age in OLETF rats compared with LETO rats, indicating the development of insulin resistance at this age, prior to the impairment of pancreatic β-cell function. Thereafter, serum glucose and insulin levels in OLETF rats further increased significantly with age both at fasting and after a glucose load. In very old OLETF rats past 40 weeks of age, glucose-induced insulin release was significantly lower versus age-matched LETO rats, indicating the progression of diabetes to an insulin-deficient form.

OLETF and LETO rats consumed approximately 26 to 30 and 23 g of food per day, respectively, and the mean food intake remained nearly the same until the end of the study. Since troglitazone did not alter food consumption in either strain of rats, the daily dosage of troglitazone in OLETF rats was approximately 52 to 60 mg per rat, which is equivalent to 135 mg/kg body weight (at 12 weeks of age) to 85 mg/kg body weight (at 70 weeks of age), and in LETO rats it was approximately 45 mg per rat, equivalent to 143 mg/kg body weight (at 12 weeks of age) to 85 mg/kg body weight (at 70 weeks of age). It is therefore calculated that OLETF rats weighing approximately 670 g (44 weeks old) consumed about 30 g troglitazone-containing diet, which is equivalent to 3.134 kg dry food (11,269.9 kcal) containing 6,269 mg troglitazone per day in 70-kg humans. Although the dose of troglitazone in OLETF and LETO rats in the present study is 10 to 15 times greater than the therapeutic dose in humans (400 mg/d), the total caloric intake per day is about 5 times greater in the rat versus the human, and the dose of troglitazone in the present study is routinely used to elicit an insulin-sensitizing response in diabetic mice and rats.

Compared with LETO rats, the abnormalities in fasting serum glucose, insulin, and TG in OLETF rats were already apparent at 6 weeks of age, whereas serum FFA and cholesterol were increased versus LETO rats at 12 and 28 weeks of age, respectively. It has been reported that the TG content of the islets is not significantly different in 6-week-old OLETF rats versus LETO rats, but it is significantly high at 12 weeks of age compared with LETO rats.[28] Taken together, it is conceivable that the impairment of insulin action appears before the onset of abnormal lipid metabolism in OLETF rats. Indeed, we have found that an insensitivity to insulin appears before the onset of abnormal lipid metabolism and precedes hyperglycemia in OLETF rats after the cessation of a 16-week treatment with an α-glucosidase inhibitor.[17] However, in contrast, previous studies have suggested that a primary increase in plasma TG and FFA levels can lead to hyperglycemia by multiple converging mechanisms.[29,30] In the course of diabetes in OLETF rats, hypertriglyceridemia might result in significant TG storage in the islets, which subsequently inhibits glucose-induced insulin secretion by reducing glucokinase activity in the islets,[28] leading to an insulin-deficient form.

Our results also show that troglitazone reduces the insulin resistance, as indicated by the marked decrease in fasting serum insulin and the improved insulinogenic index in troglitazone-treated OLETF rats. Troglitazone substantially decreased serum glucose at basal and after an intravenous glucose load, and it maintained insulin secretory function until the end of the study at 70 weeks of age. The initial step in the development of type 2 diabetes is a defect in insulin action. The resulting resistance to insulin-mediated glucose disposal can be compensated for by an overproduction of insulin. Our present observation indicates that troglitazone administration not only inhibits the onset of diabetes but also reverses diabetes in OLETF rats. Since the adverse effects of hyperglycemia per se on insulin secretory function have been well described,[31,32] the prevention of hyperglycemia by troglitazone would be expected to protect the β cell from these adverse effects. In addition, the improvement of β cell function by troglitazone treatment may also relate to a reduction in TG and FFA levels.

TZDs are known to activate the peroxisome proliferator–activated receptor gamma (PPARγ), which is expressed primarily in adipose tissue.[33] The binding of TZDs to PPARγ correlates well with many of their in vivo activities, including activation of lipoprotein lipase (LPL)[34] and attenuation of hyperglycemia.[21,33] Troglitazone decreased circulating TG, FFA, and total cholesterol levels in OLETF rats without altering the total weight of white adipose tissue as was observed in patients with type 2 diabetes[12-15] and in obese Zucker rats.[10] Since FFAs are known to reduce peripheral glucose utilization (insulin resistance) and to promote hepatic glucose overproduction,[29] and since under some conditions, these higher levels may contribute to the inhibition of glucose-stimulated insulin release,[35] the reduced concentrations could have contributed to the improved insulin secretory response and the normal glycemic response to an IVGTT after treatment. Moreover, an amelioration of insulin resistance causes an increase in the antilipolytic effects of insulin, resulting in an attenuation of FFA release from adipose tissue. Indeed, troglitazone almost completely normalized the abnormally elevated serum FFA levels in OLETF rats. In addition, troglitazone might have enhanced the

rate of TG removal by reducing insulin resistance in peripheral tissues and increasing the action of insulin on LPL. Taken together, the decrease in serum TG might be elicited by a series of alterations of insulin resistance that include a decrease of serum insulin and an enhancement of insulin responsiveness in adipose tissue. However, a recent study has shown that troglitazone increases the number of small adipocytes and decreases the number of large adipocytes in white adipose tissue, which normalizes the increased expression of tumor necrosis factor alpha and higher levels of plasma lipids.[36] Moreover, Burant et al[37] have shown that troglitazone can alter glucose and lipid metabolism in an experimental model of lipodystrophy in mice, suggesting that important regulation of PPARγ can occur in the absence of fat.

The etiology of diabetes and histologic degeneration of β cells in OLETF rats appear somewhat different versus human type 2 diabetes. The morphologic features noted in OLETF rats are (1) a sporadic onset of changes of islets in the same lobule, (2) large islets with hyperplasia of β cells in the hyperplastic stage, (3) atrophy of hyperplastic foci and loss of β cells in the later stage, (4) fibrosis and clustering of the islets in the later stage, (5) hemosiderin deposition around the islets, and (6) no amyloid deposition in the stroma during any stage.[38] In the islets of human type 2 diabetics, 3 morphologic findings have been reported, (1) islet amyloidosis, (2) islet fibrosis, and (3) changes in islet cellular composition.[39] Although amyloid deposition was not noted in OLETF rats and the histologic degeneration of β cells in OLETF rats is somewhat different from the changes in human type 2 diabetes, islet fibrosis and a loss of β cells developed with age as in human type 2 diabetics.

The pancreatic islets of untreated OLETF rats are forced to secrete more insulin to overcome the loss of normal insulin sensitivity, resulting in the impairment of pancreatic β cells at the final stage. Troglitazone administration might have spared β-cell overwork by improving peripheral insulin sensitivity, thus preventing the islets from secreting a large amount of insulin to overcome the loss of normal insulin sensitivity. Moreover, troglitazone, along with its hypoglycemic activity, greatly increased pancreatic insulin content in OLETF and LETO rats compared with untreated control rats. In addition, the histologic changes in the islets observed in untreated OLETF rats were minimal in troglitazone-treated OLETF rats. In both OLETF and LETO rats treated with troglitazone, the size and number of pancreatic islets appeared to be smaller and fewer versus the untreated control. The morphologic effect and elevation of pancreatic insulin content in troglitazone-treated rats are probably due to an alleviation of the demand for circulating insulin that, in turn, may arise from more efficient glucose utilization in treated OLETF and LETO rats. Interestingly, troglitazone ameliorated the fibrosis and derangement of the islets and greatly increased pancreatic wet weight in OLETF rats. Caloric restriction,[40] exercise training,[41] and treatment with insulin[42] or an α-glucosidase inhibitor[17] have also been shown to ameliorate fibrosis and atrophy of the islets and the exocrine pancreas, and improve the β-cell response to glucose. It is therefore likely that the loss of acini in the pancreas of untreated OLETF rats is due not to a congenital CCK-A receptor deficiency as proposed by Jimi et al,[43] but to the development and deterioration of diabetes mellitus as observed in type 1 diabetes.[44] In support of this view, the most recent studies have demonstrated that mice lacking functional CCK-A receptors[45] and CCK-deficient mice[46] have normal pancreatic weight and cellular morphology, suggesting that CCK is not essential for maintaining pancreatic function.

Several previous studies in humans have shown that troglitazone prevents the progression from impaired glucose tolerance (IGT) to type 2 diabetes.[47-49] Nolan et al[47] have first demonstrated that treatment with troglitazone 200 mg orally twice daily for 12 weeks decreases insulin resistance and improves glucose tolerance in obese subjects with either IGT or normal glucose tolerance, and suggested the usefulness of troglitazone in preventing type 2 diabetes. The effects of treatment with troglitazone in patients with IGT were further confirmed by Antonucci et al,[48] who showed that the glycemic response after a glucose load is statistically and clinically significantly improved for 80% of patients with IGT after 12 weeks of treatment with troglitazone (400 mg every morning). However, a recent randomized clinical trial to test the possibility of preventing or delaying the onset of type 2 diabetes in high-risk individuals with elevated fasting plasma glucose and IGT discontinued the trial of troglitazone because of liver toxicity.[49]

In summary, this study has shown that troglitazone reduces the insulin resistance and maintains the postglycemic insulin response at a normal level, and thus inhibits the development of insulin insensitivity and frank diabetes, in the OLETF rat up to 70 weeks of age. Although it is difficult to transfer the present observations made in a particular animal model to the human situation, our long-term study in a rodent model of type 2 diabetes mellitus, the OLETF rat, shows that pharmacologic intervention with troglitazone almost completely prevents the development of diabetes when used before the onset of diabetes, and it also reverses hyperglycemia, hyperinsulinemia, and dyslipidemia even when used after the onset of diabetes.

ACKNOWLEDGMENT

We thank Kayoko Ariyoshi for technical assistance.

REFERENCES

1. Pfeifer MA, Halter JB, Porte D Jr: Insulin secretion in diabetes mellitus. Am J Med 70:578-588, 1981

2. Olefsky JM: Pathogenesis of insulin resistance and hyperglycemia in non–insulin-dependent diabetes mellitus. Am J Med 79:1-7, 1985 (suppl 3B)

3. Beck-Nielsen H, Vaag A, Damsbo P, et al: Insulin resistance in skeletal muscles in patients with NIDDM. Diabetes Care 15:418-429, 1992

4. DeFronzo RA: The triumvirate: β-Cell, muscle and liver. A collusion responsible for NIDDM. Diabetes 37:667-687, 1988

5. American Diabetes Association: Standards of medical care for patients with diabetes mellitus. Diabetes Care 20:S5-S13, 1997 (suppl 1)

6. Fujita T, Sugiyama Y, Taketomi S, et al: Reduction of insulin resistance in obese and/or diabetic animals by 5-[4-(1-methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878, U-63,287, ciglitazone), a new antidiabetic agent. Diabetes 32:804-810, 1983

7. Fujiwara T, Yoshioka S, Yoshioka T, et al: Characterization of new oral antidiabetic agent CS-045. Studies in KK and ob/ob mice and Zucker fatty rats. Diabetes 37:1549-1558, 1988

8. Lee MK, Miles PD, Khoursheed M, et al: Metabolic effects of

troglitazone on fructose-induced insulin resistance in the rat. Diabetes 43:1435-1439, 1994

9. Khoursheed M, Miles PD, Gao KM, et al: Metabolic effects of troglitazone on fat-induced insulin resistance in the rat. Metabolism 44:1489-1494, 1995

10. Sreenan S, Struris J, Pugh W, et al: Prevention of hyperglycemia in the Zucker diabetic fatty rat by treatment with metformin or troglitazone. Am J Physiol 271:E742-E747, 1996

11. Iwamoto Y, Kuzuya T, Matsuda A, et al: Effect of new oral antidiabetic agent CS-045 on glucose tolerance and insulin secretion in patients with NIDDM. Diabetes Care 14:1083-1086, 1991

12. Suter SL, Nolan JJ, Wallace P, et al: Metabolic effects of new oral hypoglycemic agent CS-045 in NIDDM subjects. Diabetes Care 15:193-203, 1992

13. Nolan JJ, Ludvik B, Beerdsen P, et al: Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. N Engl J Med 331:1188-1193, 1994

14. Mimura K, Umeda F, Hiramatsu S, et al: Effects of a new oral hypoglycemic agent (CS-045) on metabolic abnormalities and insulin resistance in type 2 diabetes. Diabet Med 11:685-691, 1994

15. Kumar S, Boulton AJ, Beck-Nielsen H, et al: Troglitazone, an insulin action enhancer, improves metabolic control in NIDDM patients. Diabetologia 39:701-709, 1996

16. Kawano K, Hirashima T, Mori S, et al: Spontaneous long-term hyperglycemic rat with diabetic complications. Otsuka Long-Evans Tokushima fatty (OLETF) strain. Diabetes 41:1422-1428, 1992

17. Yamamoto M, Jia DM, Fukumitsu K, et al: Metabolic abnormalities in the genetically obese and diabetic Otsuka Long-Evans Tokushima fatty rat can be prevented and reversed by α-glucosidase inhibitor. Metabolism 48:347-354, 1999

18. Ishida K, Mizuno A, Min Z, et al: Which is the primary etiologic event in Otsuka Long-Evans Tokushima fatty rats, a model of spontaneous non–insulin-dependent diabetes mellitus, insulin resistance or impaired insulin secretion? Metabolism 44:940-945, 1995

19. Okauchi N, Mizuno A, Zhu M, et al: Effects of obesity and inheritance on the development of non–insulin-dependent diabetes mellitus in Otsuka-Long-Evans-Tokushima fatty rats. Diabetes Res Clin Pract 29:1-10, 1995

20. Miles PD, Higo K, Romeo OM, et al: Troglitazone prevents hyperglycemia-induced but not glucosamine-induced insulin resistance. Diabetes 47:395-400, 1998

21. Berger J, Bailey P, Biswas C, et al: Thiazolidinediones produce a conformational change in peroxisomal proliferator-activated receptor-gamma: Binding and activation correlate with antidiabetic actions in db/db mice. Endocrinology 137:4189-4195, 1996

22. Davoren PR: The isolation of insulin from a single cat pancreas. Biochim Biophys Acta 63:150-153, 1962

23. Bondar RJL, Mead DC: Evaluation of glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* in the hexokinase method for determining glucose in serum. Clin Chim Acta 20:586-590, 1974

24. Morgan CR, Lazarow AL: Immunoassay of insulin: Two antibody system. Diabetes 12:115-126, 1963

25. Labarca C, Paigen K: A simple, rapid and sensitive DNA assay procedure. Anal Biochem 102:344-352, 1980

26. Aizawa T, Taguchi N, Sato Y, et al: Prophylaxis of genetically determined diabetes by diazoxide: A study in a rat model of naturally occurring obese diabetes. J Pharmacol Exp Ther 275:194-199, 1995

27. Moran TH, Katz LF, Plata-Salaman CR, et al: Disordered food intake and obesity in rats lacking cholecystokinin A receptors. Am J Physiol 274:R618-R625, 1998

28. Man ZW, Zhu M, Noma Y, et al: Impaired β-cell function and deposition of fat droplets in the pancreas as a consequence of hypertriglyceridemia in OLETF rat, a model of spontaneous NIDDM. Diabetes 46:1718-1724, 1997

29. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 45:3-10, 1996

30. Mingrone G, Henriksen FL, Greco AV, et al: Triglyceride-induced diabetes associated with familial lipoprotein lipase deficiency. Diabetes 48:1258-1263, 1999

31. Rossetti L, Giaccari A, DeFronzo RA: Glucose toxicity. Diabetes Care 13:610-629, 1990

32. Leahy JL, Bonner-Weir S, Weir GC: β-Cell dysfunction induced by chronic hyperglycemia. Diabetes Care 15:442-453, 1992

33. Spiegelman BM: PPAR-γ: Adipogenic regulator and thiazolidine receptor. Diabetes 47:507-514, 1998

34. Stewart MW, Laker MF, Dyer RG, et al: Lipoprotein compositional abnormalities and insulin resistance in type II diabetic patients with mild hyperlipidemia. Arterioscler Thromb 13:1046-1052, 1993

35. Sako Y, Grill VE: A 48-hour lipid infusion in the rat time-dependently inhibits glucose-induced insulin secretion and B cell oxidation through a process likely coupled to fatty acid oxidation. Endocrinology 127:1580-1589, 1990

36. Okuno A, Tamemoto H, Tobe K, et al: Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. J Clin Invest 101:1354-1361, 1998

37. Burant CF, Sreenan S, Hirano K, et al: Troglitazone action is independent of adipose tissue. J Clin Invest 100:2900-2908, 1997

38. Jimi A, Miyasaka K, Shinozaki H, et al: Morphological changes in the pancreatic islets of the OLETF rat, in Shima K (ed): Obesity and NIDDM: Lessons from the OLETF Rat. New York, NY, Elsevier Science, 1999, pp 29-36

39. Gepts W, Lecompte PM: The pancreatic islets in diabetes. Am J Med 70:105-115, 1981

40. Okauchi N, Mizuno A, Yoshimoto S, et al: Is caloric restriction effective in preventing diabetes mellitus in the Otsuka Long Evans Tokushima fatty rat, a model of spontaneous non–insulin-dependent diabetes mellitus? Diabetes Res Clin Pract 27:97-106, 1995

41. Shima K, Shi K, Mizuno A, et al: Exercise training has a long-lasting effect on prevention of non–insulin-dependent diabetes mellitus in Otsuka-Long-Evans-Tokushima fatty rats. Metabolism 45: 475-480, 1996

42. Ishida K, Mizuno A, Sano T, et al: Effect of timely insulin administration on pancreatic B-cells of Otsuka-Long-Evans-Tokushima-fatty (OLETF) strain rats; an animal model of non–insulin dependent diabetes mellitus (NIDDM). Horm Metab Res 27:389-402, 1995

43. Jimi A, Kojiro M, Miyasaka K, et al: Apoptosis in the pancreas of genetically diabetic rats with a disrupted cholecystokinin (CCK-A) receptor gene. Pancreas 14:109-112, 1997

44. Gepts W: Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 14:619-633, 1965

45. Kopin AS, Mathes WF, McBride EW, et al: The cholecystokinin-A receptor mediates inhibition of food intake yet is not essential for the maintenance of body weight. J Clin Invest 103:383-391, 1999

46. Lacourse KA, Swanberg LJ, Gillespie PJ, et al: Pancreatic function in CCK-deficient mice: Adaptation to dietary protein does not require CCK. Am J Physiol 276:G1302-G1309, 1999

47. Nolan JJ, Ludvik B, Beerdsen P, et al: Improvement of glucose tolerance and insulin resistance in obese subjects treated with troglitazone. N Engl J Med 331:1188-1193, 1994

48. Antonucci T, Whitcomb R, McLain R, et al: Impaired glucose tolerance is normalized by treatment with the thiazolidinedione troglitazone [published erratum appears in Diabetes Care 21:678, 1998]. Diabetes Care 20:188-193, 1997

49. The Diabetes Prevention Program Research Group: The Diabetes Prevention Program; design and methods for a clinical trial in the prevention of type 2 diabetes. Diabetes Care 22:623-634, 1999