Bezafibrate on Lipids and Glucose Metabolism in Obese Diabetic Otsuka Long-Evans Tokushima Fatty Rats

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Type 2 diabetes is caused by insulin resistance and β-cell dysfunction. The Otsuka Long-Evans Tokushima Fatty (OLETF) rat is an established animal model of human type 2 diabetes that exhibits chronic and slowly progressive hyperglycemia and hyperlipidemia and is accompanied by progressive fibrosis in the islets. The aim of the present study was to examine whether worsening of hyperglycemia, insulin resistance, and histologic alterations of the islets in OLETF rats is related to hyperlipidemia by treating these animals with a lipid-lowering drug, bezafibrate. The bezafibrate-treated groups of OLETF and their control counterpart Long-Evans Tokushima Otsuka (LETO) rats received a bezafibrate-rich diet (150 mg/100 g normal chow) for 16 weeks, from 12 to 28 weeks of age, while the other groups of rats received standard rat chow. Bezafibrate treatment significantly reduced serum triglyceride (TG) and free fatty acid (FFA) levels, suppressed the increase in islet size, and inhibited the expression of α-smooth muscle actin, a marker for activated pancreatic stellate cells that are involved in the fibrosis of the pancreas, in the islets in OLETF rats, but had no influences on food intake, body weight gain, abdominal adipose depots, and pancreatic insulin content in both strains of rats. Although bezafibrate significantly reduced circulating lipid levels and suppressed the increase in insulin secretion evaluated by area under the curve (AUC) analysis in response to an intravenous glucose tolerance test (IVGTT) until the end of the experiment, improvement of insulin resistance was observed only for the first 8 weeks after the onset of bezafibrate treatment. These results suggest that dyslipidemia is not responsible for the reduced insulin sensitivity, but the impairment of glucose tolerance is the primary defect in the OLETF rats, although improvement of dyslipidemia suppressed histologic alterations in the islets and temporally improved insulin resistance.

TYPE 2 DIABETES is caused by insulin resistance and β-cell dysfunction. The Otsuka Long-Evans Tokushima Fatty (OLETF) rat is an established animal model of human type 2 diabetes.[1] OLETF rats exhibit a late-onset of chronic and slowly progressive hyperglycemia and show innate polyphagia, which causes rapid body weight gain, resulting in hyperinsulinemia, hypertriglyceridemia, and hyperglycemia. Insulin resistance appears at 12 to 24 weeks of age, and overt diabetes develops at 20 to 30 weeks of age. At later than 40 weeks of age, OLETF rats become hypoinsulinemic, and they also have defects in insulin secretion.[1-3] Histologically, OLETF rat shows the progressive fibrosis in the pancreas. After 20 weeks of age, the fibrosis and enlargement of the islets become prominent, and the islets are clustered by connective tissues.[1,4] After 40 weeks of age, the islets are replaced by connective tissues. After 70 weeks of age, the pancreas is extremely atrophic, and the tissue is replaced by fatty and connective tissue. Both the number and size of islets are decreased significantly.[1-4] Thus, the genetically obese-hyperglycemic OLETF rat has many similarities with human type 2 diabetes, and its diabetic syndrome is characterized by a high degree of insulin resistance.[1-3,5,6]

Hypertriglyceridemia is known to be a feature of obesity-related type 2 diabetes, but the pathoetiologic significance of this association is obscure. In our previous studies,[2,3] we found that abnormalities in fasting serum glucose, insulin, and triglyceride (TG) values in the OLETF rats are already apparent at 6 weeks of age compared with the control counterpart Long-Evans Tokushima Otsuka (LETO) rats, whereas those in serum concentrations of free fatty acid (FFA) and cholesterol increase above those in LETO rats at 12 and 28 weeks of age, respectively.[3] Administration of α-glucosidase inhibitor, acarbose, to OLETF rats reduced serum glucose and insulin levels and improved hyperlipidemia.[2] After the cessation of a 16-week treatment with an α-glucosidase inhibitor, however, impairment of insulin action (insulin resistance) appeared before the onset of abnormal lipid metabolism and preceded hyperglycemia.[2] We have also demonstrated that activation of peroxisome proliferator-activated receptor (PPAR)-γ by chronic oral administration of troglitazone in the OLETF rats reduces insulin resistance and maintains the postglycemic insulin response at a normal level, and thus, inhibits the development of insulin insensitivity, hyperlipidemia, severe histopathologic changes of pancreatic islets, and frank diabetes up to 70 weeks of age.[3] These results suggest that the impairment of insulin action appears before the onset of abnormal lipid metabolism in the OLETF rats. In contrast to these observations, several previous studies have revealed that a primary increase in plasma TG and FFA levels can lead to hyperglycemia by multiple converging mechanisms.[7-10] Man et al[9] revealed that hypertriglyceridemia causes significant TG stores in the islets of OLETF rats, which subsequently inhibits glucose-induced insulin secretion. Moreover, Lee et al[10] have reported that plasma FFA begins to increase progressively 2 weeks before hyperglycemia in Zucker diabetic fatty (ZDF) rats, and that correlations exist among plasma FFA and glucose concentrations and islet triacylglycerol contents.

Because pancreatic β cells express the PPAR-α, β, and γ isoforms[11] and because PPAR-γ agonist, troglitazone, not only

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prevented, but also dramatically improved insulin sensitivity, glucose tolerance, and morphologic changes and reduced insulin resistance and hyperlipidemia in OLETF,[3] we undertook the present study to examine whether treatment with PPAR-α agonist, bezafibrate, has similar preventive effects on insulin resistance and morphologic changes of the islets in the OLETF rats to those observed with troglitazone. Indeed, some studies have reported that bezafibrate reduces serum lipids levels, insulin resistance, and fasting serum glucose levels in non-obese type 2 diabetic patients.[12-15]

MATERIALS AND METHODS

Animals and Diet

Spontaneously diabetic OLETF rats and their counterpart control LETO rats were kindly supplied by the Tokushima Research Institute, Otsuka Pharmaceutical, Tokushima, Japan. Rats were maintained in a temperature (23°C ± 20°C)- and humidity (55% ± 15%)-controlled room with a 12-hour light/dark cycle (lights on at 7 AM). The animals were provided standard rat chow ad libitum consisting of (as a percentage of calories) 61% carbohydrate, 26% protein, and 13% fat with cellulose (wt/wt) (3.596 kcal/g diet; Oriental Yeast, Tokyo, Japan) and tap water. The rats were maintained according to the ethical guidelines of our institution, and the experimental protocol was approved by the animal welfare committee at our university.

Administration of Bezafibrate

Standard rat chow was pulverized to a fine powder, and bezafibrate (a generous gift from Kissei Pharmaceutical, Tokyo, Japan) was added and thoroughly mixed to a final concentration of 150 mg/100 g food. The drug-chow powder mixture was reconstituted into pellets with a normal appearance. Chow for control rat was prepared in a similar fashion, but without the addition of bezafibrate.

OLETF and LETO rats at 12 weeks of age consumed approximately 28 and 22 g of food/day, respectively, and the average food intake remained nearly the same until the end of this study. Because bezafibrate did not alter food consumption in both strains of rats, the daily dosage of bezafibrate taken by the OLETF rats was approximately 42 mg/rat, which is equivalent to 105 mg/kg body weight (at 12 weeks of age) to 70 mg/kg body weight (at 28 weeks of age), and that by the LETO rats was approximately 33 mg/rat, which is equivalent to 110 mg/kg body weight (at 12 weeks of age) to 66 mg/kg body weight (at 28 weeks of age). The dose of bezafibrate given to OLETF and LETO rats in the present study was lower or similar to that used to reduce serum lipids in hyperlipidemic rat,[16,17] but approximately 10 times higher than that used to improve insulin sensitivity in rats.[12]

Experimental Protocol

All animals were fed standard rat chow until 12 weeks of age and then randomly divided into 2 groups (the start of the experiment). The treated groups of rats were maintained on bezafibrate-rich chow from 12 weeks of age until the end of the study at 28 weeks. The control groups of rats received standard rat chow free of bezafibrate.

Rats in all groups were allowed free access to food and water throughout the study. 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 average food intake was estimated as the amount of food consumed per cage.

At 6 weeks of age, after an overnight fast, the rats were intraperitoneally (IP) injected with 50 mg/kg body weight sodium pentobarbital, and blood samples were collected from the jugular vein for measurement of insulin, glucose, TG, and FFA. At 12, 16, 20, 24, and 28 weeks of age, an intravenous glucose tolerance test (IVGTT) was performed after an overnight fast. Animals were weighed before the experiments, and anesthesia was induced using sodium pentobarbital. A bolus dose of 200 mg/kg body weight glucose was injected into the right jugular vein immediately after blood sampling for measurement of serum concentrations of insulin, glucose, TG, and FFA. Blood samples were collected again from the left jugular vein at 5, 10, 30, and 60 minutes for measurements of serum concentrations of glucose and insulin. After IVGTT, the abdomen was quickly opened, and retroperitoneal, mesenteric, and epididymal white adipose depots were dissected and weighed. The pancreas was excised and cleared of lymph nodes and fat. Portions of each pancreatic tissue with similar anatomic orientation were used for histologic examination and biochemical determination.

A portion of the pancreatic tissue was homogenized in saline using a motor-driven 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 standing was used for DNA assay. Insulin was extracted by a modified method of Davoren.[18]

Quantitative Analysis for Pancreatic Islets

A portion of the pancreatic tissue was fixed overnight in 10% formaldehyde solution for hematoxylin and eosin staining, and a quantitative evaluation of the islet size in the pancreatic specimen after pharmacologic intervention with or without bezafibrate was performed using an Axiophot microscope (Carl Zeiss, Eching, Germany) connected to an interactive image analysis system (IBAS, Carl Zeiss). Ten nonoverlapping fields per pancreatic specimen of hematoxylin and eosin staining ($n = 6$) were randomly selected at a × 100 magnification. The area of islet (μm^2) was determined by IBAS image analysis system.

Immunohistochemistry

Histologic changes were evaluated by immunostaining for α-smooth muscle actin (α-SMA), a marker for activated pancreatic stellate cells (PSCs) that are involved in the fibrosis of endocrine and exocrine pancreas.[4,19]

Paraffin-embedded pancreatic tissue sections were prepared on glass slides. The sections were treated with graded alcohol solutions and incubated for 15 minutes in 3% H_2O_2 to block endogenous peroxidase activities. Nonspecific staining was blocked by incubating with bovine normal serum for 10 minutes at room temperature. These sections were incubated with mouse antihuman α-SMA antibody (Dako, Carpinteria, CA) at a dilution 1:50 at room temperature for 30 minutes. Bound antibodies were detected with the peroxidase-labeled streptavidin-biotin (LSAB) method using a commercially available kit (LSAB Kit; Dako), and all procedures were performed as recommended by the manufacturer. These sections were then stained with diaminobenzidine (DAB). Counterstaining was performed with Mayer's hematoxylin, and the sections were mounted.

Assays

Serum glucose concentrations were determined by the glucose-oxidase method using a glucose kit (Glucose-E reagent; International Reagents, Kobe, Japan).[20] Insulin concentrations in the serum and pancreatic homogenates were measured by radioimmunoassay using the double-antibody method[21] with a commercially available radioimmunoassay kit (ShionoRIA; Shionogi Pharmaceutical, Osaka, Japan) using crystalline rat insulin as a reference standard. Serum TG and FFA concentrations were determined by enzymatic colorimetric methods using commercially available kits (Wako Pure Chemical, Tokyo, Japan). DNA concentrations in pancreatic homogenates were determined by the method of Labarca and Paigen[22] using the fluorescent dye

Table 1. Effect of Bezafibrate Treatment on Body Weight, Adipose Tissue Weight, and Pancreatic Insulin Content in OLETF and LETO Rats at 28 Weeks of Age

Parameters	LETO		OLETF	
	L-Cont	L-BF	O-Cont	O-BF
Body weight (g)	461 ± 10	451 ± 13	593 ± 9†	602 ± 23†
Food intake (g/d)	21.2 ± 0.4	21.3 ± 0.3	28.7 ± 0.4†	29.4 ± 0.5†
Serum lipid concentrations				
TG (mmol/L)				
6 weeks of age*	0.42 ± 0.03		1.0 ± 0.06†	
12 weeks of age*	0.32 ± 0.04		1.2 ± 0.08†	
28 weeks of age	0.63 ± 0.09‡	0.43 ± 0.06	1.75 ± 0.06†‡	1.23 ± 0.21†§
FFA (g/L)				
6 weeks of age*	0.150 ± 0.013		0.145 ± 0.020	
12 weeks of age*	0.153 ± 0.016		0.229 ± 0.022†	
28 weeks of age	0.174 ± 0.031	0.094 ± 0.016‡§	0.236 ± 0.135	0.120 ± 0.025†‡§
Total adipose tissue (g/rat)	21.1 ± 2.3	18.5 ± 1.4	56.7 ± 3.0†	52.3 ± 3.3†
(mg/g body weight)	45.2 ± 4.2	40.9 ± 2.8	94.6 ± 3.3†	88.7 ± 1.2†
Pancreatic insulin content				
(nmol/pancreas)	20.1 ± 1.1	21.5 ± 0.6	26.8 ± 1.4†	25.9 ± 1.6†
(nmol/mg DNA)	2.66 ± 0.20	2.71 ± 0.23	4.06 ± 0.20†	3.58 ± 0.15†

NOTE. Values are the mean ± SEM of 6 to 8 rats.

Abbreviations: L-Cont, control LETO rats; L-BF, bezafibrate-treated LETO rats; O-Cont, control OLETF rat; O-BF, bezafibrate-treated OLETF rats.

*Data from both the untreated control and the bezafibrate-treated groups before the start of bezafibrate administration (at 12 weeks of age) were pooled as the control.

†Significant difference *v* L-Cont at the same age.

‡Significant difference *v* respective baseline at 12 weeks of age.

§Significant difference *v* respective control.

H-33258 (Hoechst, AG, Frankfurt, Germany) and calf thymus DNA (Type I; Sigma Chemical, St Louis, MO) as a standard. All samples were assayed at least in duplicate.

Insulin resistance was calculated by homeostasis model assessment insulin resistance (HOMA-IR) with the following formula: fasting insulin (mU/mL) × fasting glucose (mmol/L)/22.5, as described by Matthews et al.[23] With such a method, high HOMA scores denote low insulin sensitivity (insulin resistance).[23] Because the HOMA-IR is only based on fasting insulin and glucose concentrations, there are limitations of the HOMA-IR method. However, HOMA-IR value is revealed to be highly correlated with insulin resistance calculated by the minimal model approach in subjects with varying degrees of glucose tolerance.[24,25]

Statistical Analysis

Results are presented as the mean ± SEM. Differences between groups were tested for statistical significance using analysis of variance (ANOVA) followed by Tukey's test. A P value less than .05 denoted a statistically significant difference. The same statistical procedure was used to analyze the results of serum glucose and insulin concentrations using area under the curve (AUC) analysis.

RESULTS

Food Consumption and Body Weight

OLETF rats at 6 weeks of age consumed more food than LETO rats. The average food intake of OLETF rats gradually increased up to 28.5 ± 0.5 g/rat/d at 12 weeks of age and remained at nearly the same levels until the end of the study. Food intake in the control LETO rats also increased up to 22.5 ± 0.3 g/rat/d at 12 weeks of age and remained at nearly the same levels until the end of the study (Table 1). Bezafibrate treatment did not influence food intake in both strains of rats. Based on the food intake, the daily dosage of bezafibrate taken by the OLETF rats was estimated to be approximately 42 mg/rat, which is equivalent to 105 to 70 mg/kg body weight/day, and that by the LETO rats was approximately 33 mg/rat, which is equivalent to 110 to 66 mg/kg body weight/day.

OLETF rats at 6 weeks of age were already significantly heavier than LETO rats at the corresponding age (229.0 ± 5.0 g *v* 190.0 ± 2.0 g, $P < .01$). The body weight of both strains of rats increased progressively with age, although the weight gain in OLETF rats was significantly greater than that in LETO rats. Addition of bezafibrate to the diet had no significant influence on weight gain in both strains of rats (Table 1).

Fasting Serum Levels of TG and FFA

Fasting serum TG levels at 6 weeks of age in OLETF rats were already significantly higher than those in LETO rats (0.94 ± 0.08 *v* 0.38 ± 0.02 mmol/L, $P < .01$) and increased progressively with age (Table 1). Addition of bezafibrate to the diet from 12 weeks of age markedly decreased serum TG levels from 1.21 ± 0.08 mmol/L at 12 weeks of age to 0.75 ± 0.06 mmol/L at 16 weeks of age ($P < .01$ *v* at 12 weeks of age). After 16 weeks of age, serum TG levels in bezafibrate-treated OLETF rats slightly increased, but were significantly lower than those in the control OLETF rats at the corresponding age. In LETO rats, fasting serum TG levels gradually increased with age, and bezafibrate prevented the age-related TG increase (Table 1).

The fasting serum FFA levels in OLETF rats were nearly the same as those in LETO rats at 6 weeks of age (0.144 ± 0.010 *v* 0.138 ± 0.018 g/L, not significant [NS]), but were signifi-

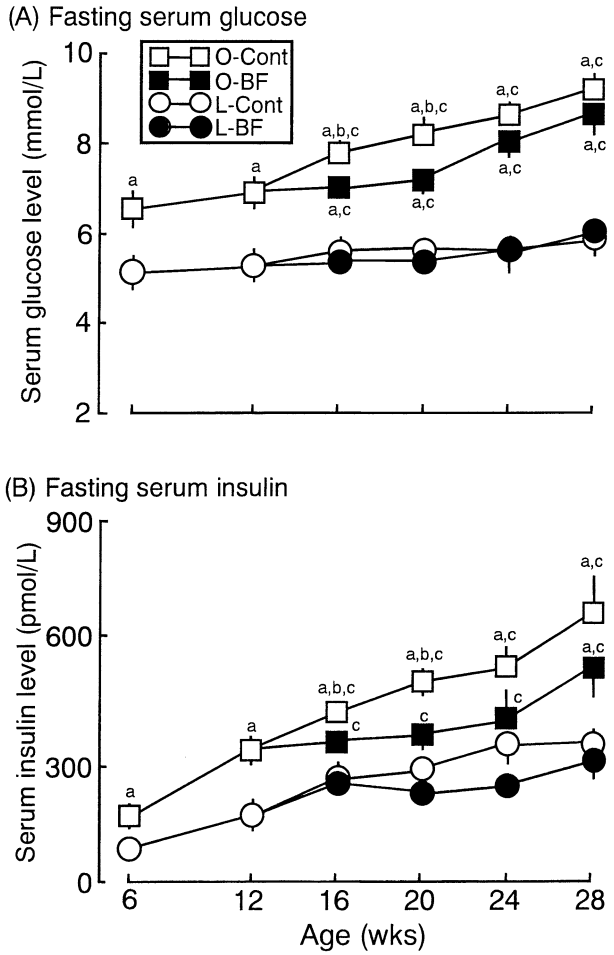

Fig 1. Serial changes of (A) fasting serum glucose and (B) insulin levels in the untreated control and bezafibrate-treated OLETF (□, ■) and LETO rats (○, ●). Control rats received a standard rat chow free of bezafibrate until the end of the study (○, □), whereas bezafibrate-treated rats received a bezafibrate-rich diet from 12 to 28 weeks of age (●, ■). Results are the mean ± SEM of 6 to 8 rats. O-Cont, control OLETF rat; O-BF, bezafibrate-treated OLETF rats; L-Cont, control LETO rats; L-BF, bezafibrate-treated LETO rats. [a]Significant difference *v* control LETO rats (L-Cont) at the same age; [b]significant difference *v* bezafibrate-treated OLETF rats (O-BF) at the same age; and [c]significant difference *v* bezafibrate-treated LETO rats (L-BF) at the same age.

cantly higher than those in LETO rats at 12 weeks of age (0.22 ± 0.02 *v* 0.12 ± 0.01 g/L, $P < .01$) and remained at higher levels until the end of this study at 28 weeks of age. Supplementing the diet with bezafibrate significantly decreased serum FFA levels in both OLETF and LETO rats (Table 1).

Serum Insulin and Glucose Response to IVGTT

Fasting serum glucose (Fig 1A) and insulin (Fig 1B) levels in OLETF rats were significantly higher than those in LETO rat at 6 weeks of age, before the start of this experiment, and increased progressively with age.

In the control untreated OLETF rats, the glycemic response to an IVGTT gradually increased with age (Fig 2A). Addition of bezafibrate to the diet from 12 weeks of age prevented the age-dependent increase in serum glucose levels at fasting until 20 weeks of age (12 weeks of age *v* bezafibrate-treated OLETF rats at 20 weeks of age, 6.7 ± 0.3 *v* 7.4 ± 0.2 mmol/L, NS) (Fig 1A). Bezafibrate significantly suppressed the total glucose response represented by the area of glucose curve (AUC) until 24 weeks of age (Fig 3A).

Fasting and the AUC of insulin response exhibited progressive elevations as the OLETF rats increased in age (Figs 1B, 2B, and 3B). Although suppressive effects of bezafibrate on the age-dependent increase in basal serum insulin levels were observed only for the first 8 weeks after the onset of treatment (from 14 to 24 weeks of age) (Fig 1B), bezafibrate almost completely prevented the age-related increases in the AUC of insulin response to an IVGTT until the end of the study at 28 weeks of age (Fig 3B).

In the control LETO rats, fasting serum glucose concentrations showed a tendency to increase with age (Fig 1A), whereas fasting serum insulin concentrations significantly increased from 108 ± 12 pmol/L at 6 weeks of age to 225 ± 23 pmol/L at 12 weeks of age and further increased with age (Fig 1B). Bezafibrate had no significant influences on fasting and postglycemic serum glucose (Figs 1A, 2A, and 3A) and insulin levels (Figs 1B, 2B, and 3B) in the LETO rats.

HOMA-IR

HOMA-IR in OLETF rats at 6 weeks of age was already significantly higher than that in LETO rats and progressively increased with age (Fig 4). Although there are limitations of the HOMA-IR method, which is only based on fasting insulin and glucose concentrations, addition of bezafibrate to the diet prevented the increase in the HOMA-IR for the first 8 weeks after the onset of treatment (until 20 weeks of age). Therefore, bezafibrate treatment transiently prevented the increase in insulin resistance. HOMA-IR in the control LETO rats also showed a slight, but progressive, increase with age (Fig 4).

Abdominal Fat Weight and Pancreatic Insulin Content

Total abdominal fat weight (mesenteric, retroperitoneal, and epididymal) in the untreated control OLETF rats was almost 3 times heavier than that in the control LETO rats (Table 1). Supplementing the diet with bezafibrate showed a tendency to decrease the total adipose depots in both strains of rats, although not statistically significant.

The pancreatic insulin content in the control OLETF rats was significantly higher than that in the control LETO rats. Administration of bezafibrate tended to decrease the pancreatic insulin content in OLETF rats, whereas it tended to increase in LETO rats. However, the differences were not statistically significant compared with that in the respective control rats (Table 1).

Histologic Change

The islets of the untreated control OLETF rats were larger and had irregular boundaries (Fig 5A). Bezafibrate treatment greatly suppressed the increase in islet size (Fig 5B).

Quantitative analysis of pancreatic islet size in OLETF and LETO rats by using an IBAS imaging analysis system revealed an age-related increase in islet size (Table 2). The islets of the

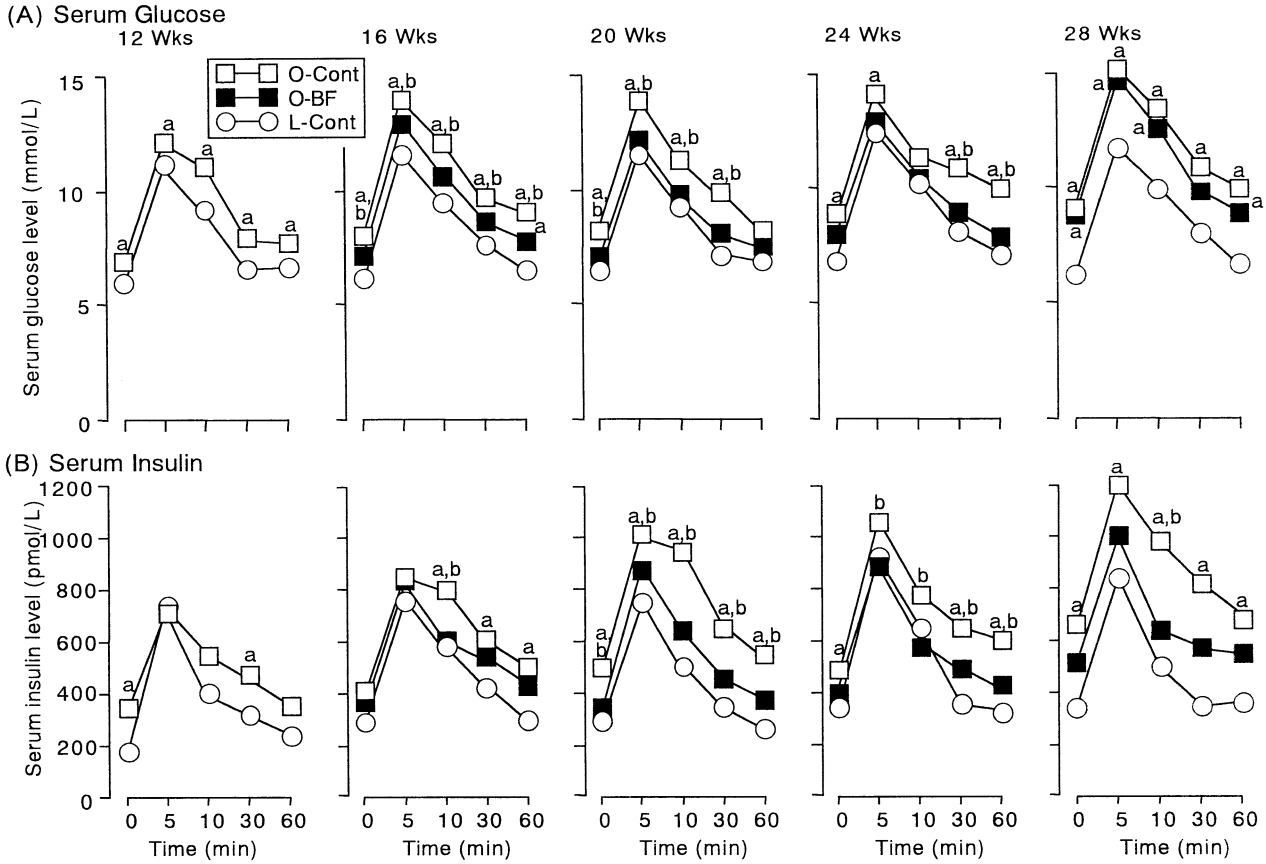

Fig 2. Serial changes in (A) serum glucose and (B) insulin responses to an IVGTT (0.2 g/kg body weight) in OLETF (□, ■) and LETO rats (○). Control rats received a standard rat chow free of bezafibrate until the end of the study (○, □), whereas bezafibrate-treated rats received a bezafibrate-rich diet from 12 to 28 weeks of age (■). Results are expressed as the mean of 6 to 10 rats. Because there was no difference in serum glucose and insulin levels between the untreated control and the bezafibrate-treated groups before the start of bezafibrate administration, the data from both groups were pooled as the control to make the figure simple. In the LETO rats, the data only from the untreated control LETO rats is shown, because there were no differences in serum glucose and insulin levels between the untreated control and the bezafibrate-treated LETO rats. O-Cont, control OLETF rat; O-BF, bezafibrate-treated OLETF rats; L-Cont, control LETO rats. [a]Significant difference *v* L-Cont at corresponding time point; [b]significant difference *v* O-BF at corresponding time point.

untreated control OLETF rats were larger and had irregular boundaries. In addition, α-SMA was strongly expressed in the enlarged islets of the untreated control OLETF rats (Fig 5A). Distribution of α-SMA-positive cells closely correlated with the area of connective tissue proliferation. No significant histologic changes, such as enlargement of islets and connective tissue proliferation, were observed in the control LETO rats, and bezafibrate had no influences on the pancreas of the LETO rats. Administration of bezafibrate to OLETF rats reversed the size of islets to that in LETO rats (Table 2). Moreover, bezafibrate significantly suppressed the connective tissue proliferation and the expression of α-SMA in the islets of the OLETF rats (Fig 5B). In the pancreas of bezafibrate-treated OLETF rats, α-SMA immunoreactivity was seen only in the vessel walls (Fig 5B).

DISCUSSION

Type 2 diabetes is a syndrome characterized by insulin resistance and defective insulin secretion.[26,27] Hypertriglyceridemia and/or elevated serum FFA levels induces insulin resistance in the liver and peripheral tissue.[28-30] It is not known, however, whether the increased serum lipids are responsible for the reduced insulin sensitivity or whether the increased serum lipids are secondary to the metabolic derangement due to insulin resistance.

In the present study, treatment with bezafibrate lowered fasting serum TG and FFA levels until the end of the experimental period, as were observed after treatment with PPAR-γ agonist, troglitazone[3] and suppressed connective tissue proliferation and α-SMA expression in the islets of the OLETF rats. However, the improvement of insulin resistance and glucose metabolism was quite small compared with that observed after troglitazone treatment[3] and was only transient for the first 8 weeks after the onset of treatment. Although the mechanism of the transient effects of bezafibrate on glucose metabolism (escape phenomenon or secondary failure) is unclear, these observations suggest a possibility that the improvement of glucose metabolism is not due to an improvement of lipid metabolism, but the impairment of insulin action is the primary defect in the OLETF rats. Indeed, abnormalities in fasting serum glucose

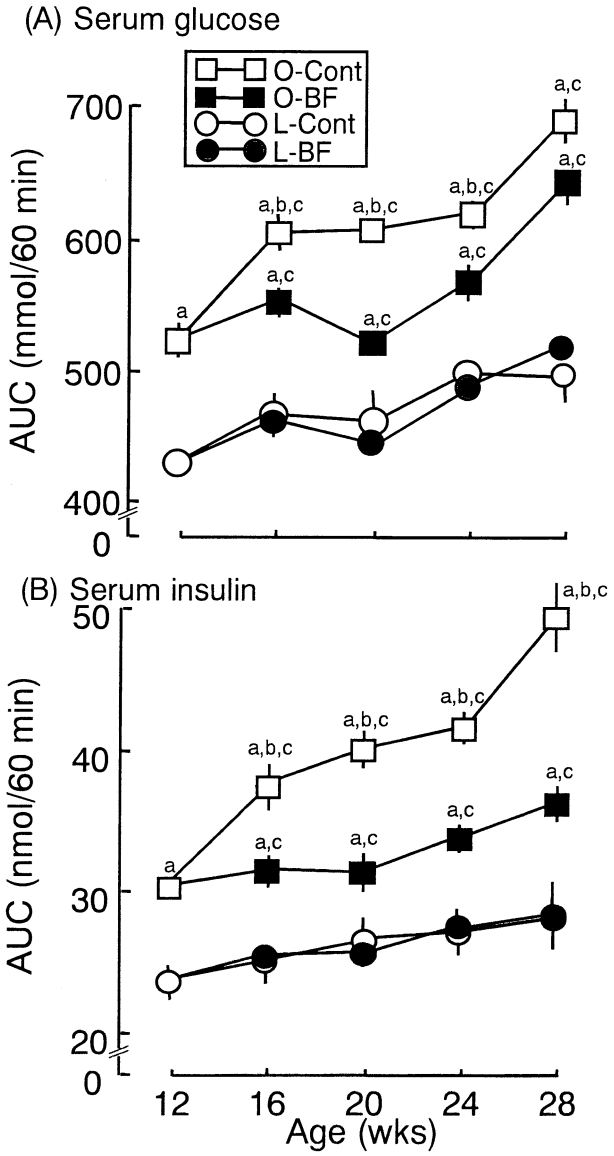

Fig 3. Serial changes of the (A) area of glucose and (B) insulin curves in response to an IVGTT in the untreated control and bezafibrate-treated OLETF (□, ■) and LETO rats (○, ●). Control rats received a standard rat chow free of bezafibrate until the end of the study (□, ○), whereas bezafibrate-treated rats received a bezafibrate-rich diet from 12 to 28 weeks of age (■, ●). Results are the mean ± SEM of 6 to 8 rats. O-Cont, control OLETF rat; O-BF, bezafibrate-treated OLETF rats; L-Cont, control LETO rats; L-BF, bezafibrate-treated LETO rats. [a]Significant difference *v* L-Cont at the same age; [b]significant difference *v* O-BF at the same age; [c]significant difference *v* L-BF at the same age.

and insulin levels in OLETF rats were already apparent at 6 weeks of age when serum FFA levels were still similar to those in the control LETO rat (Table 1). Moreover, we found that administration of α-glucosidase inhibitor, acarbose, to OLETF rats not only reduces serum glucose and insulin levels, but also improves hyperlipidemia, and that the increase in serum glucose levels and insulin resistance after the cessation of treatment with an α-glucosidase inhibitor precedes the increase in plasma TG and FFA in OLETF rats.[2] These results suggest that the increase in serum lipids is secondary to derangement of glucose metabolism in OLETF rats. In support of this view, Harmon et al[16] have demonstrated in the ZDF rat that bezafibrate treatment does not prevent the increase in fasting plasma glucose nor the associated decrease in insulin mRNA levels, and that treatment with phlorizin, a drug that reduces plasma glucose, but has no influence on lipid levels, prevents hyperglycemia and preserves insulin mRNA levels without preventing hypertriglyceridemia. More recently, Koishi et al[31] have demonstrated that complete lack of hyperlipidemia in the KK/San mice does not improve the diabetic phenotype and indicated that the correction of diabetic dyslipidemia may not improve glucose tolerance.

In contrast, however, improvement of dyslipidemia is often shown to reduce insulin resistance and improve glucose tolerance.[12,13,15,32-36] Panz et al[33] have suggested that the pathogenesis of lipoatrophic diabetes mellitus (LDM) is related primarily to abnormal regulation of lipids rather than glucose metabolism by demonstrating an improvement of β-cell function, a decrease in insulin resistance, and the attainment of normal glucose homeostasis in parallel with progressive reductions in serum concentrations of TG and nonesterified fatty acids (NEFA) after administration of bezafibrate. Matsui et al[12] have also shown that bezafibrate improves insulin sensitivity in rats fed the high-fructose plus lard diet by normalizing the fatty acid composition of skeletal-muscle TG. Moreover, the increase in plasma FFA levels in ZDF rats precedes the increase in plasma glucose levels by 2 weeks.[10]

Although we have no explanation for these discrepancies of

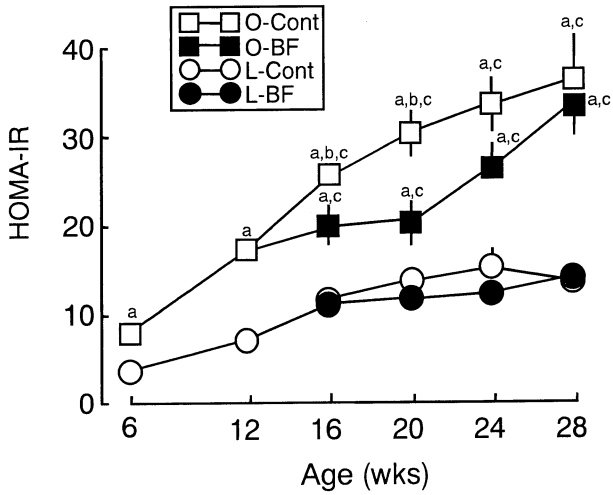

Fig 4. Serial changes of HOMA-IR in the untreated control and bezafibrate-treated OLETF (□, ■) and LETO rats (○, ●). Control rats received a standard rat chow free of bezafibrate until the end of the study (□, ○), whereas bezafibrate-treated rats received a bezafibrate-rich diet from 12 to 28 weeks of age (■, ●). Results are the mean ± SEM of 6 to 8 rats. O-Cont, control OLETF rat; O-BF, bezafibrate-treated OLETF rats; L-Cont, control LETO rats; L-BF, bezafibrate-treated LETO rats. [a]Significant difference *v* L-Cont at the same age; [b]significant difference *v* O-BF at the same age; [c]significant difference *v* L-BF at the same age.

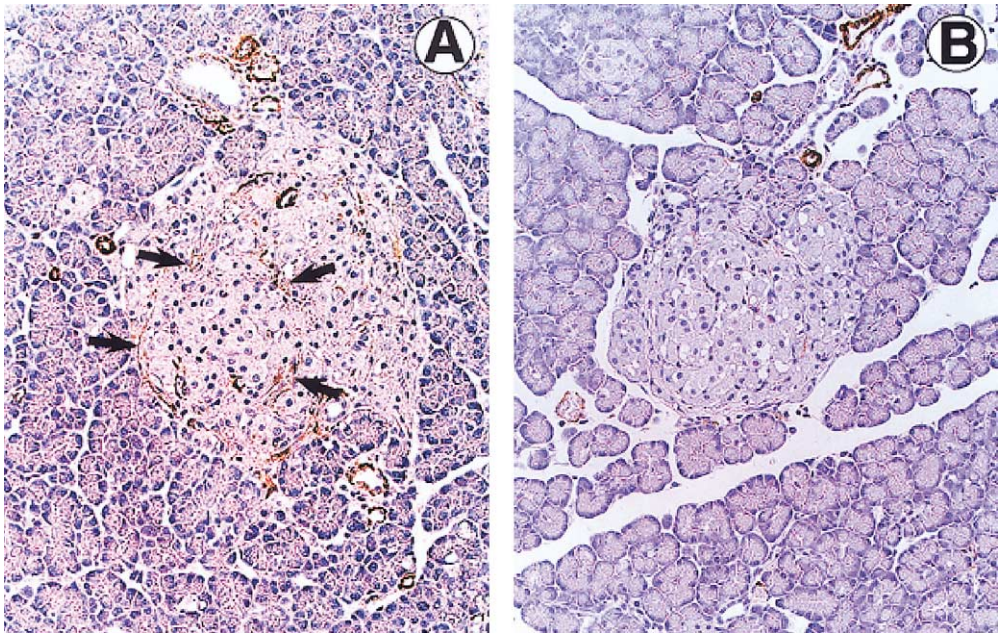

Fig 5. Representative immunohistochemistry of α-SMA expression in the pancreas at 28 weeks of age. In the (A) untreated control OLETF rats, islet was enlarged with irregular boundaries, and α-SMA was strongly expressed in the enlarged islets. In the (B) bezafibrate-treated OLETF rats, the expression of α-SMA in the islet was completely suppressed, and α-SMA immunoreactivity was seen only in the vessel walls. Original magnification × 50.

the effects of bezafibrate on glucose metabolism, it seems possible that bezafibrate may have different effects on humans[13,15,28,29,32] from those on rodents.[10,12] Differences of species of animals used, ZDF,[10] Sprague-Dawley rats,[12] and OLETF rats (present study) and the doses and duration of bezafibrate administration might be other possible explanations for the discrepancies. In fact, if we brought the present study to an end at 16 or 20 weeks of age, 4 to 8 weeks after the commencement of bezafibrate administration, we might have concluded that bezafibrate improves glucose tolerance and insulin sensitivity by lowering serum levels of TG and FFA (Figs 1, 2, 3, and 4).

Bezafibrate is not a pure PPAR-α agonist, but activates PPAR-α, β, and γ with a comparable EC_{50} value.[37] There is a possibility therefore that bezafibrate influences glucose homeostasis through the activation of PPAR-β and/or PPAR-γ subtypes in adipocytes. It is also conceivable that PPAR-α, PPAR-β, and PPAR-γ are differently activated by bezafibrate depending on the doses used and the duration of administration. The highly selective PPAR-γ agonist, troglitazone, reduced the insulin resistance and maintained the postglycemic response at a normal level without changing body weight and adipose depots in OLETF rats up to 70 weeks of age.[3] Thus, bezafibrate was less effective than troglitazone for preventing and improving insulin resistance (Jia et al[3] *v* present study). On the other hand, the selective PPAR-α agonists, such as WY14,643[38] and fenofibrate[39] are shown to decrease body weight and visceral fat and improve insulin action. However, bezafibrate had no influences on body weight gain and total abdominal adipose depots in OLETF rats. Taken together, the results of the present

Table 2. Effect of Bezafibrate Treatment on Pancreatic Islet Size in OLETF and LETO Rats

	LETO		OLETF	
Age (wk)	L-Cont	L-BF	O-Cont	O-BF
6	3,669 ± 45	ND	4,917 ± 410*	ND
12	8,395 ± 1,255†	ND	13,025 ± 787*†	ND
28	7,710 ± 1,330†	6,130 ± 216†	27,592 ± 4,438*†‡	7,378 ± 1,620†‡§

NOTE. Values are the mean ± SEM of 5 rats per group. Size of pancreatic islets in LETO and OLETF rats was evaluated by an IBAS image analysis system and indicated as μm^2.

Abbreviations: L-Cont, control LETO rats; L-BF, bezafibrate-treated LETO rats; O-Cont, control OLETF rats; O-BF, bezafibrate-treated OLETF rats; baseline, 12 weeks of age; ND, not determined.

*Significant difference *v* L-Cont at the same age.

†Significant difference *v* 6 weeks of age in the same strain of rats.

‡Significant difference *v* 12 weeks of age in the same strain of rats.

§Significant difference *v* respective control without bezafibrate.

study indicate that multi-PPAR agonist, bezafibrate, has smaller effects on both PPAR-α and PPAR-γ compared with the respective selective agonists in this strain of rats fed standard rat chow.

The pancreatic β cells in OLETF rats are forced to secrete more insulin to overcome the loss of normal insulin sensitivity, resulting in the islet to enlarge in a hyperplastic stage.[1,4] Although improvement of insulin resistance by bezafibrate treatment was observed only at early stage after the commencement of the treatment, the AUC of insulin response to an IVGTT (Fig 3B) and the islet size evaluated by an IBAS image analysis system in the bezafibrate-treated OLETF rats were greatly improved until the end of the study at 28 weeks of age (Table 2). In addition, administration of bezafibrate greatly suppressed connective tissue proliferation and the expression of α-SMA, a marker for activated PSCs that are involved in the fibrosis of the pancreas.[4,19] Recently, we have also found that bezafibrate greatly inhibits the infiltration of inflammatory cell and suppresses the expression of tumor necrosis factor (TNF)-α and interleukin (IL)-1β in the exocrine pancreas.[40] Indeed, many studies have indicated that bezafibrate interferes with the response of inflammatory cytokines and induces apoptosis of monocyte-derived macrophages.[41,42] These results suggest that bezafibrate reduces insulin resistance and improves β-cell function by reducing circulating lipid levels and probably accumulation of TG in the islets. Further long-term studies are required to examine whether the histologic changes observed in the present study after bezafibrate administration for 16 weeks would slow the ultimate pancreatic failure seen in older OLETF rats.[2,3]

In summary, administration of bezafibrate to OLETF rats not only improved the hyperlipidemia and suppressed the connective tissue proliferation in the islets, but also delayed the progression of hyperglycema and insulin resistance. Because the improvement of dyslipidemia and histologic alterations in the islets was observed until the end of the study at 28 weeks of age, and because the improvement of insulin resistance and glucose metabolism was quite small and transient, it is reasonable to conclude that dyslipidemia is not responsible for the reduced insulin sensitivity, and that the impairment of glucose tolerance is the primary defect in the OLETF rats. Although it is difficult to transfer the present observation made in a particular animal model to the human situation, bezafibrate may have some beneficial effects on insulin resistance and islet morphology in patients with obese type 2 diabetes.

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