

Peroxisome Proliferator–Activated Receptor (PPAR)- α Activation Prevents Diabetes in OLETF Rats

Comparison With PPAR- γ Activation

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Lipid accumulation in nonadipose tissues is closely related to the development of type 2 diabetes in obese subjects. We examined the potential preventive effect of peroxisome proliferator–activated receptor (PPAR)- α and PPAR- γ stimulation on the development of diabetes in obese diabetes-prone OLETF rats. Chronic administration of a PPAR- α agonist (0.5% [wt/wt] fenofibrate) or a PPAR- γ agonist (3 mg \cdot kg⁻¹ \cdot day⁻¹ rosiglitazone) completely prevented the development of glycosuria. Pancreatic islets from untreated OLETF rats underwent sequential hypertrophy and atrophy, which was completely prevented by chronic fenofibrate treatment. In contrast, rosiglitazone treatment did not affect islet hypertrophy at earlier stages but prevented β -cell atrophy at later stages. Fenofibrate treatment decreased body weight and visceral fat, whereas rosiglitazone treatment increased body weight. Despite the opposite effects on adiposity, both drugs were equally effective in improving insulin actions in skeletal muscle. Furthermore, both drugs significantly decreased the triglyceride content in the soleus muscle and pancreatic islets. The present study demonstrates that the PPAR- α agonist fenofibrate prevents the development of diabetes in OLETF rats by reducing adiposity, improving peripheral insulin action, and exerting beneficial effects on pancreatic β -cells. *Diabetes* 52:2331–2337, 2003

Increasing evidence suggests that lipid accumulation in nonadipose tissues, such as skeletal muscle and pancreatic islet, is causally related to the development of type 2 diabetes in obese individuals (1,2). Peroxisome proliferator–activated receptors (PPARs) are members of the superfamily of nuclear transcription factors that regulate lipid metabolism (3). Thiazolidinedione (TZD), a high-affinity ligand for PPAR- γ , is now widely used as an insulin-sensitizing drug (4) and has been shown

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2-DG, 2-deoxy-D-[U-¹⁴C]glucose; FFA, free fatty acid; PPAR, peroxisome proliferator–activated receptor; TZD, thiazolidinedione.

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to reduce fat accumulation in skeletal muscle (5). TZD has also been suggested to reduce fat accumulation in other nonadipose tissues such as islets and heart (6). However, these favorable effects of TZD on nonadipose tissues may not arise directly from PPAR- γ activation in these tissues. PPAR- γ is highly expressed in adipose tissue, but its expression is low in nonadipose tissues such as skeletal muscle or pancreatic β -cells (7). Therefore, it has been suggested that TZD improves muscle insulin action and prevents apoptosis of pancreatic β -cells by sequestering lipids in adipose tissue (8) or by increasing production of adiponectin (9), an adipocytokine that has been shown to increase insulin sensitivity (10).

The role of PPAR- α in the regulation of intracellular lipid homeostasis in nonadipose tissues may be more straightforward. Normally, it is expressed in nonadipose tissues at relatively high levels compared with PPAR- γ (11). PPAR- α is a transcription factor that has been shown to upregulate fatty acid oxidative enzymes mainly in the liver (12), but recent studies have indicated that it also increases fatty acid oxidation in skeletal muscle (13). It was also reported that PPAR- α stimulators increase insulin sensitivity and reduce adiposity (14) and lipid accumulation in skeletal muscle (5). The expression of PPAR- α and enzymes of fatty acid oxidation is markedly reduced in the fat-laden dysfunctional islets of obese prediabetic Zucker diabetic fatty (*fa/fa*) rats (15). It is unknown, however, whether PPAR- α stimulators can prevent β -cell destruction and diabetes in diabetes-prone animals. The present study was therefore undertaken to examine the potential preventive effects of PPAR- α stimulation on the development of diabetes in Otsuka Long Evans Tokushima Fatty (OLETF) rats, an animal model of obesity and diabetes.

RESEARCH DESIGN AND METHODS

Animals. Male OLETF rats and their lean nondiabetic counterparts, Long-Evans Tokushima Otsuka (LETO) rats, were supplied at 4 weeks of age by the Otsuka Pharmaceutical Company (Tokushima, Japan). The rats were maintained at an ambient temperature (22 \pm 1°C) with 12:12-h light-dark cycles and free access to water and rat food. All procedures were approved by the Institutional Animal Care and Use Committee at the Asan Institute for Life Sciences in Seoul, Korea.

The effects of fenofibrate and rosiglitazone administration on the development of diabetes, pancreatic islet morphology, and adiposity. We first examined the effect of administration of fenofibrate and rosiglitazone on the development of diabetes in OLETF rats. A total of 54 OLETF rats were divided randomly into three groups ($n = 18$ per group) at 12 weeks of age. Rats were kept on standard rat food or rat food supplemented

with 0.5% (wt/wt) fenofibrate (Abbott Laboratories, Abbott Park, IL) or 3 mg \cdot kg $^{-1}$ \cdot day $^{-1}$ of rosiglitazone (GlaxoSmithKline Pharmaceuticals, Philadelphia, PA) for 28 weeks. Urinary glucose was monitored weekly with Diastix (Amex, Tokyo). Individual rats were diagnosed as diabetic on the basis of positive glycosuria.

At 40 weeks of age, the rats were killed after being anesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg). Additional OLETF rats with or without drug treatment were killed at 9, 18, and 27 weeks of age ($n = 4$ per group). LETO rats at 9, 18, 27, and 40 weeks of age ($n = 4$ per group) were used as controls. The pancreatic tissues were stained with hematoxylin and eosin. To detect insulin, the paraffin sections were incubated with a guinea pig anti-insulin polyclonal antibody (Biomed, Foster, CA), followed by the avidin-biotin peroxidase complex method of visualization.

Measurement of β -cell mass. β -Cell mass was measured according to the method of Suzuki et al. (16). Briefly, pancreatic weight was measured and four cross-sections of the pancreas were obtained randomly. The areas of the β -cells and pancreas were determined by planimetry using Image Tool for Windows, version 1.28 (University of Texas Health Science Center, San Antonio, TX). The β -cell mass (in milligrams) was determined by pancreatic weight \times (the area showing insulin positivity/the area of pancreas)^{3/2}.

Evaluation of abdominal adiposity. Computerized tomography (CT; GE Medical Systems, Milwaukee, WI) was performed as described previously (17) on two representative rats from each group at 27 weeks of age.

The effects of fenofibrate and rosiglitazone administration on metabolic parameters and insulin sensitivity. A separate study was performed to examine the effects of fenofibrate and rosiglitazone on metabolic parameters and insulin sensitivity. Starting at 12 weeks of age, OLETF rats were given a diet without or with 0.5% (wt/wt) fenofibrate or 3 mg \cdot kg $^{-1}$ \cdot day $^{-1}$ rosiglitazone ($n = 6$ per group).

Experimental protocols. At 18 weeks of age, animals were prepared for glucose clamp experiments as previously described (18,19). Six hours before the clamps (7:00 A.M.), food was removed from the cage and catheters were placed. At \sim 1:00 P.M., a blood sample was collected for measurement of plasma glucose, triglycerides, free fatty acids (FFAs), glycerol, insulin, and leptin concentrations. This was followed by a 2-h hyperinsulinemic-euglycemic clamp (clamp period) in which human insulin (Velosulin; Novo-Nordisk, Gentofte, Denmark) was continuously infused at a rate of 86 pmol \cdot kg $^{-1}$ \cdot min $^{-1}$. Blood samples were collected at 10-min intervals for the immediate measurement of plasma glucose, and 25% dextrose was infused at variable rates to clamp plasma glucose at basal levels. To estimate whole-body and skeletal muscle insulin-stimulated glucose fluxes, D-[3-³H]glucose (New England Nuclear, Boston, MA) was infused at a rate of 0.3 μ Ci/min throughout the clamps, and a bolus of 2-deoxy-D-[U-¹⁴C]glucose (2-DG) (20 μ Ci) was injected at the 75-min time point. Blood samples (60 μ l) were taken at 0, 10, 20, 40, 60, 70, 77, 80, 85, 90, 95, 100, 105, 110, and 120 min relative to the start of the insulin infusion to measure plasma D-[3-³H]glucose and 2-DG concentrations. At the end of the clamp, the rats were anesthetized and soleus muscles were frozen with aluminum tongs precooled in liquid nitrogen. The frozen samples were kept at -70°C until analysis.

Analysis of blood samples. Plasma glucose was determined using a glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH). Plasma FFA was determined by an enzymatic assay using kits from Wako Chemical (Osaka, Japan). Both plasma triglycerides and glycerol were measured using enzymatic methods (Sigma, St. Louis, MO). Plasma insulin and leptin concentrations were determined by radioimmunoassay (Linco, St. Louis, MO). Plasma [³H]glucose and [¹⁴C]-2-DG radioactivities were measured in duplicate by deproteinizing plasma samples with saturated BaOH₂ and 5.5% ZnSO₄, drying to eliminate tritiated water, and counting for [³H] and [¹⁴C] in a liquid scintillation spectrophotometer (Beckman Instruments).

Radioisotopic determination of glucose turnover. Rates of total glucose appearance and whole-body glucose uptake were determined as the ratio of the D-[³H]glucose infusion rate to the specific activity of plasma glucose during the final 40 min of the clamps.

Measurement of muscle 2-DG uptake and [³H]glucose incorporation into muscle glycogen. Free and phosphorylated 2-DG were separated by ion-exchange chromatography using an ion-exchange resin (AG1-X8; Bio-Rad Laboratories, Richmond, CA). Radioactivity was determined using a liquid scintillation spectrophotometer. From this measurement, the measurement of plasma glucose and the time course of plasma 2-DG disappearance, which reflects insulin-stimulated glucose uptake (R_g) into the tissue, were calculated (20). Incorporation of [³H]glucose into muscle glycogen was measured by the amyloglucosidase digestion method (21) to estimate glycogen synthesis during the glucose clamps.

The effect of fenofibrate and rosiglitazone on triglyceride content and fatty acid oxidation in skeletal muscle and islets. An additional 24 OLETF rats were used to examine the effects of fenofibrate and rosiglitazone on

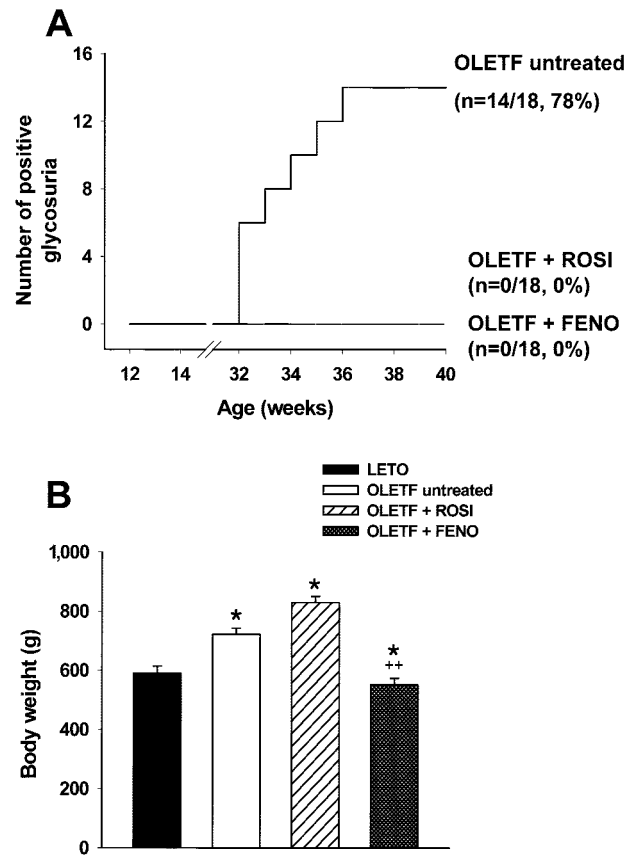


FIG. 1. The effects of rosiglitazone and fenofibrate treatment for 28 weeks on the development of positive glycosuria (A) and body weight at 40 weeks of age (B) in OLETF rats ($n = 18$ per group). FENO, fenofibrate-treated; ROSI, rosiglitazone-treated. * $P < 0.01$ vs. LETO rats; ** $P < 0.01$ vs. untreated OLETF rats.

triglyceride content and fatty acid oxidation in isolated skeletal muscle and islets. At 6 weeks of age, the rats were divided into three groups: those without treatment and those with fenofibrate or rosiglitazone treatment ($n = 8$ per group). At 12 weeks of age (after 6 weeks of drug treatment), the rats were anesthetized with an intraperitoneal injection of pentobarbital and the soleus muscle on one side was freeze-clamped in situ and removed for measurement of triglyceride concentration. The soleus muscle on the other side was removed for the measurement of fatty acid oxidation (22). Subsequently, islets were isolated by the collagenase digestion method with an injection of collagenase into the common bile duct (23). Triglyceride contents in muscle and islets were determined in duplicate using the Sigma Triglyceride (GPO-Trinder) kit as described previously (24). Palmitate oxidation was assessed by measuring [³H] water generation from 9-10-[³H] palmitate (22).

Statistical analysis. All values are expressed as means \pm SE. Statistical analysis was performed using the SPSS 10.0 program (SPSS Institute, Chicago, IL). Intergroup comparisons were performed by two-tailed unpaired Student's t test or by one-way ANOVA. For the analysis of effect on β -cell mass, the significance of the difference among the four experimental groups was assessed by two-way ANOVA using group (with four levels) and age (with three levels: 18, 27, and 40 weeks) as independent factors. Statistical significance was defined as $P < 0.05$.

RESULTS

Effects of fenofibrate and rosiglitazone on the development of diabetes, body adiposity, and visceral fat mass. Urinary glucose was detected in 14 of 18 untreated OLETF rats (78%) at 40 weeks of age. In contrast, the fenofibrate- or rosiglitazone-treated rats ($n = 18$ per group) did not show any detectable urinary glucose through 40 weeks of age (Fig. 1A). Body weight at 40 weeks of age was significantly higher in the rosiglitazone-treated rats

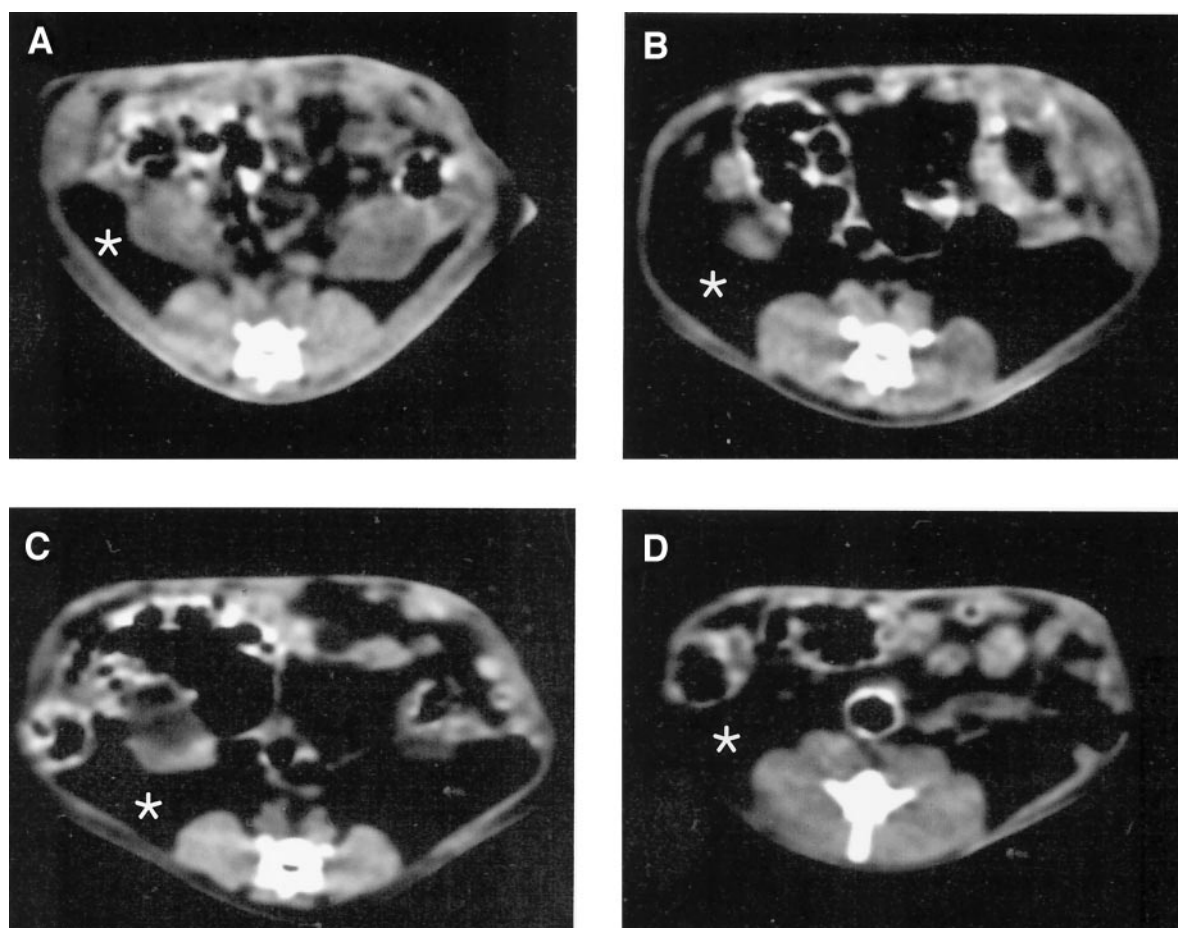


FIG. 2. The effects of fenofibrate and rosiglitazone treatments on abdominal adiposity. Drugs were given from 12 weeks of age, and abdominal computed tomography was performed in two representative rats from each group at 27 weeks of age. White stars point to the visceral fat. **A:** LETO rat; **B:** untreated OLETF rat; **C:** rosiglitazone-treated rat; **D:** fenofibrate-treated rat.

but lower in the fenofibrate-treated rats than untreated OLETF rats ($P < 0.01$ for both) (Fig. 1B). The visceral fat mass was much higher in the untreated OLETF rats than in the LETO rats (Fig. 2). Rosiglitazone treatment in OLETF rats did not significantly change the visceral fat mass, but fenofibrate treatment profoundly decreased it. Food intake was not altered by fenofibrate except in the first week, when food intake was significantly decreased by the treatment (14.7 ± 3.3 vs. 18.8 ± 1.8 g/day, $P < 0.05$). In contrast, the rosiglitazone-treated rats consumed 10–20% more food than untreated OLETF rats throughout the study period ($P < 0.05$).

Effects of fenofibrate and rosiglitazone on islet morphology. At 9 weeks of age, islets of OLETF rats appeared normal (data not shown). At 18 weeks, the islets of untreated OLETF rats were enlarged compared with the islets of LETO rats (Fig. 3). At 27 weeks, the islets of untreated OLETF rats started to be disorganized, with extensions into the surrounding exocrine tissue. At 40 weeks, insulin-positive cells were dispersed and low in number, and the contour of the islets was irregular and could not be easily delineated. The islets of rosiglitazone-treated rats at 18 weeks of age did not appear significantly different from those of untreated OLETF rats, with similar increases in insulin-positive cells. At 27 weeks, the islets of rosiglitazone-treated rats maintained a relatively normal round appearance and showed persistent increases in

insulin-positive cells. Hematoxylin and eosin staining disclosed that fibrosis and lymphocytic infiltration were milder than in the untreated group. At 40 weeks, the islets of the rosiglitazone group showed more prominent lymphocytic infiltration than islets at 27 weeks. The islets had a disorganized morphology, but the degree of atrophy was profoundly reduced compared with untreated OLETF rats, and the islet architecture remained relatively intact. The islets of fenofibrate-treated OLETF rats had a normal appearance at 18 and 27 weeks and did not show any sign of islet cell hypertrophy, destruction, or uneven distribution of insulin-positive cells. At 40 weeks of age, islets from the fenofibrate-treated group showed almost normal morphology except for a few islets with a minimal degree of hyperplasia.

Effects of fenofibrate and rosiglitazone on β -cell mass. β -Cell mass was significantly higher in untreated OLETF rats than in LETO rats at 9, 18, and 27 weeks of age ($P < 0.001$) (Fig. 4). However, β -cell mass in OLETF rats was profoundly decreased at 40 weeks of age (i.e., after the development of diabetes) to a level significantly lower than that in LETO rats ($P < 0.001$). Rosiglitazone treatment slightly increased β -cell mass at 27 weeks of age and prevented the decrease in β -cell mass in OLETF rats at 40 weeks of age. In the fenofibrate treatment group, β -cell mass remained relatively stable throughout the study period: it was significantly lower than untreated or rosigli-

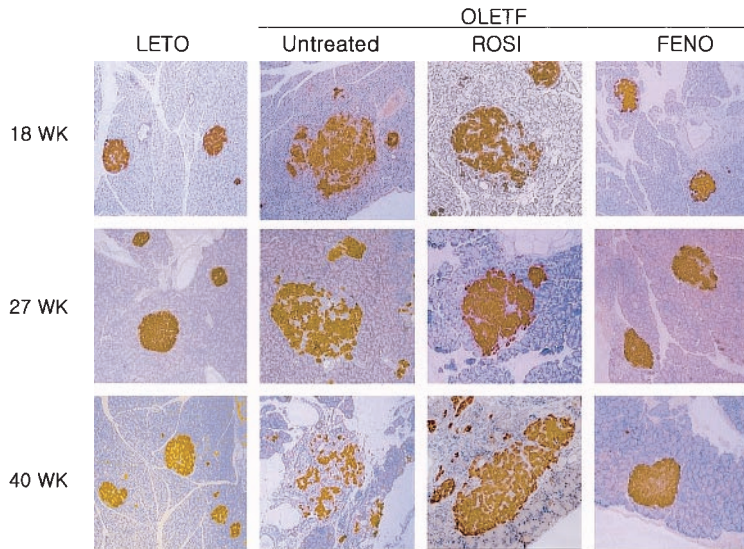


FIG. 3. Serial changes of pancreatic islets in LETO rats and OLETF rats with and without drug treatment. Binding of an anti-insulin antibody was visualized with the avidin-biotin peroxidase complex method. FENO, fenofibrate-treated; ROSI, rosiglitazone-treated.

tazone-treated OLETF rats at 27 weeks of age ($P < 0.01$), and the decrease in β -cell mass at 40 weeks of age was prevented as with rosiglitazone treatment ($P < 0.01$ vs. untreated OLETF rats).

Effects of fenofibrate and rosiglitazone on basal metabolic parameters. At 18 weeks of age (i.e., 6 weeks after the initiation of treatment), plasma insulin, FFA, triglyceride, and leptin were significantly higher in untreated OLETF rats than in LETO rats ($P < 0.05$) (Table 1). Both fenofibrate and rosiglitazone treatments decreased plasma insulin and triglyceride, but more potent effects were observed with fenofibrate. In addition, fenofibrate, but not rosiglitazone, treatment significantly decreased plasma leptin and FFA levels ($P < 0.01$). Plasma glucose was slightly higher in OLETF rats than in LETO rats and

was lowered by rosiglitazone, although these changes did not gain statistical significance. In contrast, fenofibrate significantly decreased blood glucose in OLETF rats even below the levels of LETO rats ($P < 0.01$).

Effects of fenofibrate and rosiglitazone on insulin-stimulated whole-body and muscle glucose uptake and muscle glycogen synthesis. Insulin-stimulated whole-body glucose uptake (R_d) in untreated OLETF rats was significantly lower than that in LETO rats ($P < 0.05$) (Fig. 5A). Both fenofibrate and rosiglitazone increased R_d to levels not significantly different from those of LETO rats. Similarly, insulin-stimulated glucose uptake (R_g) and glycogen synthesis in soleus muscle were significantly lower in untreated OLETF rats than in LETO rats ($P < 0.05$) and was increased by fenofibrate or rosiglitazone treatment to levels not different from those of LETO rats (Fig. 5B and C).

Triglyceride content and fatty acid oxidation in islets and skeletal muscle of rosiglitazone- and fenofibrate-treated rats. The triglyceride content was significantly higher in the islets and soleus muscle of untreated OLETF rats than in LETO rats ($P < 0.01$) (Fig. 6A and B). Both rosiglitazone and fenofibrate treatments decreased islet and muscle triglyceride content ($P < 0.01$) to levels similar to those of LETO rats. Fatty acid oxidation was significantly higher in the islets and soleus muscle of untreated OLETF rats than in those of LETO rats ($P < 0.001$ for islet, $P < 0.05$ for muscle) (Fig. 6C and D). Rosiglitazone and fenofibrate treatments further increased fatty acid oxidation in the islets and soleus muscle of OLETF rats ($P < 0.01$).

DISCUSSION

The present study demonstrates that administration of fenofibrate or rosiglitazone prevents the development of diabetes in OLETF rats. In agreement with a previous report (5), both fenofibrate and rosiglitazone decreased triglyceride accumulation in skeletal muscle and significantly improved insulin sensitivity. These results are consistent with the ideas that lipid accumulation in nonadipose tissues is causally related to the development of type 2

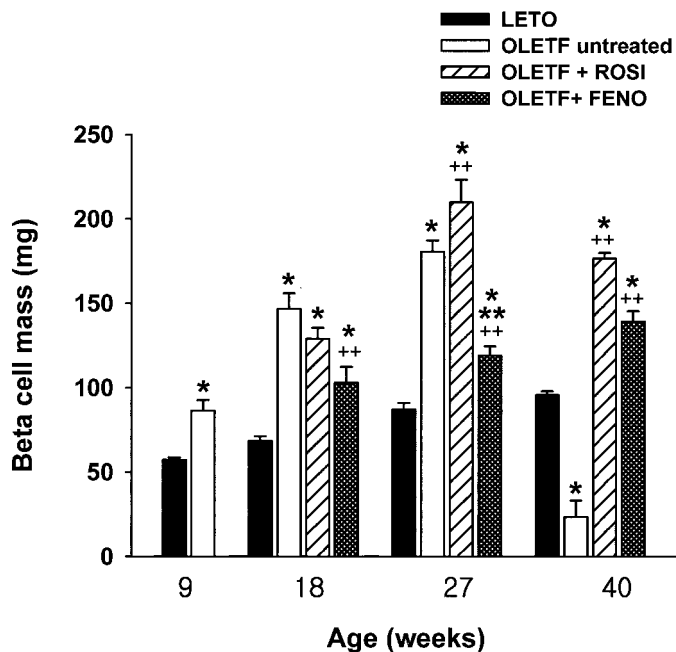


FIG. 4. Age-dependent changes in β -cell mass in the LETO and OLETF rats with and without drug treatment that were initiated at 12 weeks of age ($n = 4$ each). FENO, fenofibrate-treated OLETF rats; ROSI, rosiglitazone-treated OLETF rats. * $P < 0.01$ vs. LETO rats; ++ $P < 0.01$ vs. untreated OLETF rats; ** $P < 0.01$ vs. rosiglitazone-treated rats.

TABLE 1

Effects of rosiglitazone and fenofibrate treatments for 6 weeks on basal metabolic parameters in LETO and OLETF rats at 18 weeks of age

| | LETO rats | OLETF rats | | |
|-----------------------|-------------|--------------|---------------|----------------|
| | | Untreated | Rosiglitazone | Fenofibrate |
| Glucose (mmol/l) | 7.2 ± 0.2 | 7.7 ± 0.2 | 7.0 ± 0.3 | 6.5 ± 0.2*† |
| Insulin (pmol/l) | 570 ± 60 | 1230 ± 45‡ | 585 ± 85§ | 180 ± 15*†§ |
| Glycerol (mmol/l) | 2.4 ± 0.1 | 2.8 ± 0.3 | 1.4 ± 0.2 | 0.8 ± 0.04 |
| FFA (mmol/l) | 0.53 ± 0.08 | 0.75 ± 0.05‡ | 0.73 ± 0.08‡ | 0.47 ± 0.07*†§ |
| Triglyceride (mmol/l) | 0.78 ± 0.38 | 2.85 ± 0.37‡ | 0.67 ± 0.38 | 0.35 ± 0.37*†§ |
| Leptin (µg/l) | 7.9 ± 0.37 | 15.8 ± 3.7‡ | 14.2 ± 2.2‡ | 2.5 ± 0.7*†§ |

‡*P* < 0.05, †*P* < 0.01 vs. LETO rats; **P* < 0.01, ||*P* < 0.05 vs. untreated OLETF rats; §*P* < 0.05 vs. rosiglitazone-treated rats.

diabetes in obesity and that PPAR-α and PPAR-γ are important targets for reducing intracellular lipid accumulation.

It is currently believed that PPAR-α is involved in stimulating the β-oxidation of fatty acids, mainly in the liver (12). In contrast, PPAR-γ increases adipogenesis by stimulating adipocyte differentiation (3). This difference in drug action may explain the differential effects of these drugs on body weight and abdominal fat. Thus, fenofibrate treatment decreased body weight and visceral fat, whereas

rosiglitazone treatment increased body weight. It is conceivable that many of the metabolic effects of fenofibrate are secondary to the prevention of the weight gain. For example, reduction in adipose mass may prevent lipid accumulation in skeletal muscle or pancreatic β-cells by decreasing plasma FFA levels and FFA flux into these tissues. However, plasma glucose, insulin, and leptin levels were even lower in fenofibrate-treated OLETF rats than in LETO rats, suggesting that there are factors other than body weight.

In the case of PPAR-γ stimulation, sequestration of lipid

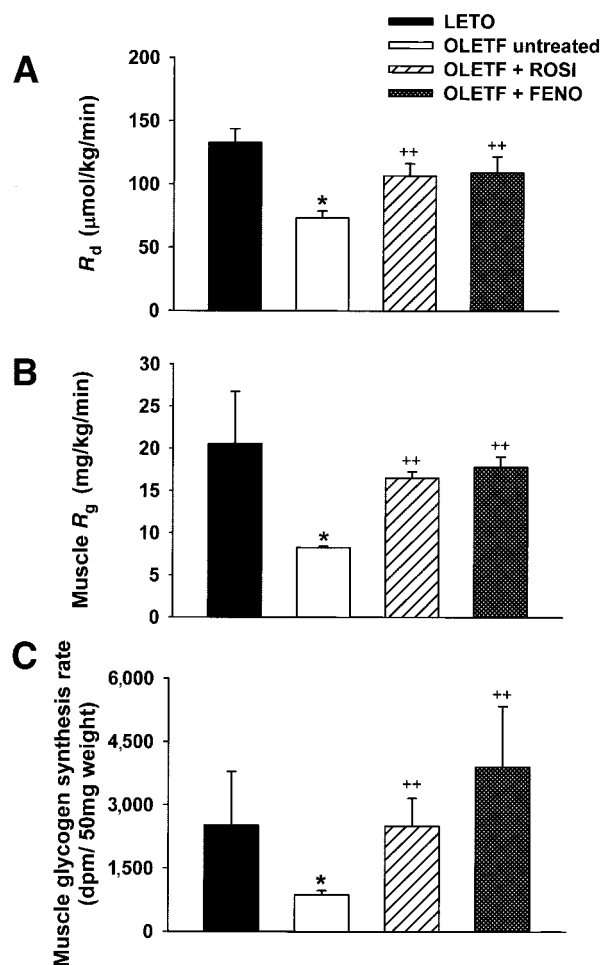


FIG. 5. Effects of fenofibrate and rosiglitazone treatments on R_a (A), insulin-stimulated glucose uptake (R_g) in soleus muscle (B), and glycogen synthesis rate in soleus muscle (C) during hyperinsulinemic-euglycemic clamps. $n = 6$ per group. Drugs were given for 6 weeks before the clamp experiments at 18 weeks of age. FENO, fenofibrate-treated OLETF rats; ROSI, rosiglitazone-treated OLETF rats. **P* < 0.05 vs. LETO rats; ***P* < 0.01 vs. untreated OLETF rats.

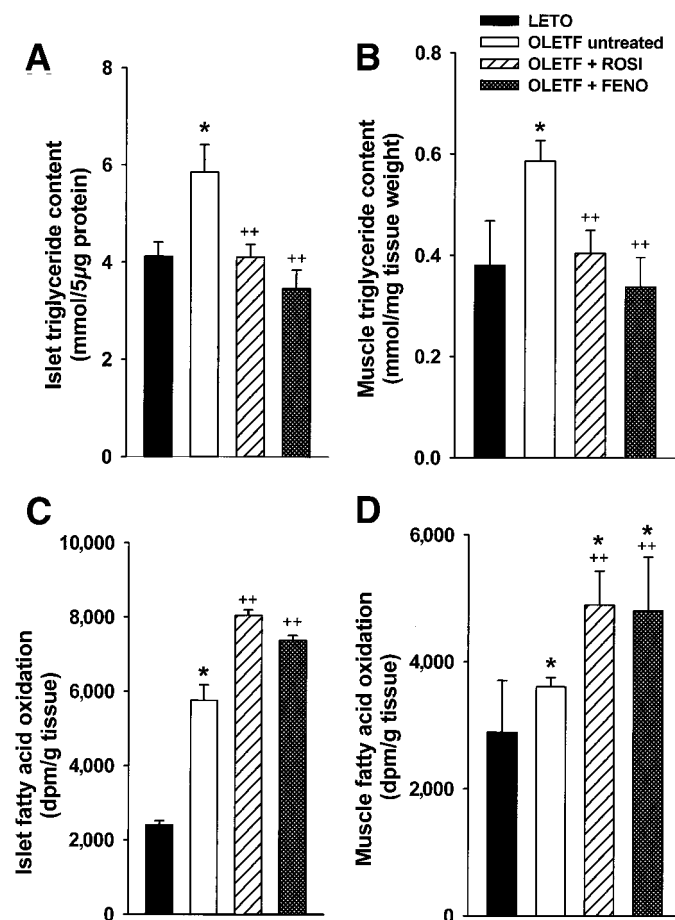


FIG. 6. Effects of rosiglitazone and fenofibrate treatments on triglyceride content and fatty acid oxidation in islets (A and C) and soleus muscle (B and D) ($n = 8$ per group). Drugs were given for 6 weeks before the experiments at 18 weeks of age. FENO, fenofibrate-treated OLETF rats; ROSI, rosiglitazone-treated OLETF rats. **P* < 0.01 vs. LETO rats; ***P* < 0.01 vs. untreated OLETF rats.

fuel into the adipose tissue might have decreased lipids in plasma and in nonadipose tissues (25); many previous studies in animals and humans have reported that PPAR- γ agonists lower plasma FFA and triglyceride levels (26). In this context, it is surprising that rosiglitazone decreased plasma triglyceride but not FFA levels in the present study. However, a recent study reported that rosiglitazone treatment of obese Zucker rats actually increased fasting plasma FFA levels via enhancing FFA mobilization (27). This effect was in marked contrast with dramatic effects of rosiglitazone to lower plasma triglyceride. Thus, the differential effects of rosiglitazone on plasma FFA and triglyceride levels in the present study are similar to those observed in obese Zucker rats.

In addition to the effects in liver and adipose tissues, recent studies have demonstrated direct stimulatory effects of PPAR- α and PPAR- γ on fat oxidation in skeletal muscle (13,28). PPAR- γ stimulation has also been shown to increase fat oxidation in islets of obese Zucker rats (6). In accordance with these previous studies, the present study demonstrated that both fenofibrate and rosiglitazone increased fatty acid oxidation in skeletal muscle and islets. However, this action of the drugs may not be the major reason for reduced tissue triglyceride contents in fenofibrate- and rosiglitazone-treated OLETF rats because fatty acid oxidation was also higher in untreated OLETF rats than in LETO rats, but tissue triglyceride levels were elevated. Therefore, the major determinant of tissue triglyceride levels may be circulating lipid levels, and fenofibrate or rosiglitazone may decrease tissue triglyceride levels primarily by decreasing plasma lipid levels.

The changes in pancreatic islets were quite different between the rats treated with fenofibrate and those treated with rosiglitazone in our study. Pancreatic islets from untreated OLETF rats underwent sequential hypertrophy and atrophy, which was completely prevented by chronic fenofibrate treatment. In contrast, rosiglitazone treatment did not affect islet hypertrophy at earlier stages but prevented β -cell atrophy at later stages. These results suggest that rosiglitazone prevents β -cell death in the islets that have already undergone hypertrophy and hyperplasia, whereas fenofibrate prevents β -cell changes from the early stages of β -cell hypertrophy and hyperplasia.

The difference in islet morphology changes between fenofibrate- and rosiglitazone-treated OLETF rats is not easily explained, because insulin sensitivity, measured using the glucose clamp at 18 weeks of age, was similarly improved by both drugs. Although we did not measure insulin secretion in response to glucose or other secretagogues, the basal plasma insulin concentration was significantly lower in the fenofibrate-treated rats than in the rosiglitazone-treated rats. This raises a possibility that, although the peripheral insulin action (R_d) measured by the glucose clamp is similar, the endogenous demand for insulin production or β -cell proliferation may be lower in the fenofibrate-treated rats. It may be possible that the liver (the major target tissue for fenofibrate action) became insulin sensitive and was responsible for the lower basal glucose in fenofibrate-treated animals. It is also possible that a difference in circulating hormone or substrate might be responsible for the differential responses of islets in fenofibrate- and rosiglitazone-treated OLETF

rats. For example, FFA concentration was much higher in the untreated OLETF rats than in the LETO rats, and fenofibrate, but not rosiglitazone, treatment decreased plasma FFA concentration in OLETF rats to the level of LETO rats. In addition to the well-known cytotoxic effect of FFA on the pancreatic islets (29), a recent study indicated that elevation of plasma FFA by lipid-heparin infusion for 2 days increased β -cell mass in rats (30). Therefore, elevated plasma FFA levels may, at least in part, be responsible for early β -cell hypertrophy and hyperplasia in the untreated and rosiglitazone-treated OLETF rats.

A concern with the measurement of islet triglyceride content is possible contamination by triglyceride from fibroblasts and/or macrophages. However, because fibrous tissues start to appear in the islets of OLETF rats from 27 weeks of age and because islet triglyceride was measured at 12 weeks of age in the present study, the increase in islet triglyceride in untreated OLETF rats should represent those in β -cells or islets per se. A previous study using Oil Red O-insulin double-staining techniques demonstrated increased deposition of fat droplets in the β -cells of OLETF rats (31), consistent with the present data.

Human PPAR- α is similar in function to rodent PPAR- α in that activation of PPAR- α leads to reduction of circulating lipids; however, other responses to PPAR- α stimulation are different (5). In rodents, peroxisome proliferation occurred in response to PPAR- α agonists and was associated with hepatic carcinogenesis in long-term studies (32). However, nonrodent species were shown to be refractory to the induction of peroxisome proliferation (33). In fact, the fibrates class of PPAR- α agonists has been safely used in humans to treat hypertriglyceridemia, and no hepatic carcinogenesis has been reported (34). Although the effect of PPAR- α activation on insulin sensitivity is inconclusive in humans, some recent clinical studies have shown improved insulin sensitivity by fibrates (35,36). It should be noted that the fenofibrate doses used in the present study ($\sim 74 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) were much higher than those clinically used in the treatment of dyslipidemia (100–250 mg/day). The issues of whether PPAR- α activation reduces adiposity and exerts a beneficial effect on pancreatic β -cells in humans warrant further investigations.

In conclusion, the present study demonstrates that the PPAR- α agonist fenofibrate prevents the development of diabetes in OLETF rats by reducing adiposity, improving peripheral insulin action, and exerting beneficial effects on pancreatic β -cells.

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