Original Article

Peroxisome Proliferator–Activated Receptor α Improves Pancreatic Adaptation to Insulin Resistance in Obese Mice and Reduces Lipotoxicity in Human Islets

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Peroxisome proliferator–activated receptor (PPAR) α is a transcription factor controlling lipid and glucose homeostasis. PPARα-deficient (−/−) mice are protected from high-fat diet–induced insulin resistance. However, the impact of PPARα in the pathophysiological setting of obesity-related insulin resistance is unknown. Therefore, PPARα$^{-/-}$ mice in an obese (*ob/ob*) background were generated. PPARα deficiency did not influence the growth curves of the obese mice but surprisingly resulted in a severe, age-dependent hyperglycemia. PPARα deficiency did not aggravate peripheral insulin resistance. By contrast, PPARα$^{-/-}$ *ob/ob* mice developed pancreatic β-cell dysfunction characterized by reduced mean islet area and decreased insulin secretion in response to glucose in vitro and in vivo. In primary human pancreatic islets, PPARα agonist treatment prevented fatty acid–induced impairment of glucose-stimulated insulin secretion, apoptosis, and triglyceride accumulation. These results indicate that PPARα improves the adaptative response of the pancreatic β-cell to pathological conditions. PPARα could thus represent a promising target in the prevention of type 2 diabetes. *Diabetes* 55:1605–1613, 2006

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ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acid; GIPR, gastrointestinal polypeptide receptor; GIR, glucose infusion rate; GLP1, glucagon-like peptide-1; GSIS, glucose-stimulated insulin secretion; IPGTT, intraperitoneal glucose tolerance test; OGTT, oral glucose tolerance test; PDX-1, pancreatic duodenal homeobox-1; PPAR, peroxisome proliferator–activated receptor.

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Type 2 diabetes is triggered by several factors such as obesity, environmental cues, and genetic predisposition. Type 2 diabetes usually develops when peripheral insulin resistance, due to ectopic fat overload in nonadipose tissues, occurs together with β-cell dysfunction characterized by progressively impaired insulin secretory capacity in response to glucose.

Peroxisome proliferator–activated receptor (PPAR) α controls several metabolic pathways of lipid and glucose metabolism. PPARα is activated by natural (fatty acids, eicosanoids) or synthetic (fibrates) ligands and is expressed in a wide range of tissues (liver, heart, kidney, and muscle). Fibrates are clinically used to treat dyslipidemia. PPARα regulates the expression of genes involved in fatty acid and lipoprotein metabolism (1). PPARα participates in the physiological response to fasting by inducing the mitochondrial β-oxidation of fatty acids released from adipose tissue, resulting in the formation of ketone bodies. Interestingly, PPARα-deficient mice develop a more severe hypoglycemia upon fasting, indicating a role for PPARα in glucose metabolism as well (2–4).

Studies performed in rodent models of insulin resistance showed that PPARα activation improves glucose homeostasis by enhancing insulin sensitivity due to a decrease in lipid content in adipose and nonadipose tissues (5–8) and/or by decreasing endogenous glucose production (7,9). PPARα is expressed in rat pancreatic islets, and PPARα agonist treatment has been reported to improve pancreatic β-cell function in insulin-resistant rodents (8,10). Surprisingly, PPARα-deficient mice are protected from diet-induced insulin resistance (11). Moreover, isolated islets from normal diet– and high-fat–fed wild-type or PPARα-deficient mice exhibit similar glucose-stimulated insulin secretion (GSIS) responses (11). Thus, the absence of peripheral insulin resistance upon high-fat feeding precluded a proper assessment of a putative role for PPARα in the pancreas.

Since the impact of PPARα deficiency on glucose homeostasis in the context of obesity-related insulin resistance has not yet been established, we first analyzed the consequences of PPARα deficiency in a genetic model of obesity-related insulin resistance. PPARα-deficient mice were crossed with leptin-deficient *ob/ob* mice, an animal model of insulin resistance and obesity. *ob/ob* mice are characterized by hyperphagia, hyperglycemia, hyperinsulinemia, and insulin resistance due to an inherited inability

to produce leptin (12). Our results show that PPARα deficiency results in the development of a more pronounced hyperglycemia with age. This aggravation of hyperglycemia is not due to alterations in peripheral insulin resistance but rather to a lack of appropriate pancreatic compensation. Second, since chronic fatty acid exposure contributes to β-cell dysfunction, the influence of PPARα activation was also investigated in human islets under conditions of high palmitate exposure. Our results show that PPARα agonist treatment reduces triglyceride accumulation and apoptosis and increases the efficacy of glucose to induce insulin release. Altogether, these data indicate that PPARα influences glucose homeostasis, in part via effects on pancreas function.

RESEARCH DESIGN AND METHODS

All animal experiments were approved by the Pasteur Institute review board, Lille, France. Leptin-deficient *ob/ob* mice deficient (PPARα$^{-/-}$ *ob/ob*) or not for PPARα (PPARα$^{+/+}$ *ob/ob*) were generated (online appendix [available at http://diabetes.diabetesjournals.org]). Their phenotypes were compared with those of nonobese mice deficient (PPARα$^{-/-}$ *OB/OB*) or not (PPARα$^{+/+}$ *OB/OB*) for PPARα.

Plasma parameters. Blood sampling was performed after a 6-h fast (8:00 A.M. to 2:00 P.M.). Glucose levels were measured on a Glucotrend 2 glucometer (Roche). For insulin and free fatty acid (FFA) determinations, blood was collected into heparinized tubes and separated by centrifugation (15 min, 1,500*g*, 4°C). Plasma insulin concentrations were measured with an enzyme-linked immunosorbent assay (Rat Insulin ELISA kit; Mercodia, Uppsala, Sweden) using rat standards. FFAs were measured enzymatically (NEFA-C kit; Wako, Dardilly, France) using oleic acid as standard.

Glucose tolerance tests. Intraperitoneal glucose tolerance tests (IPGTTs) and oral glucose tolerance tests (OGTTs) were performed on male mice after a 6-h fast (8 A.M. to 2 P.M.) (online appendix).

Hyperinsulinemic-euglycemic clamps. Hyperinsulinemic-euglycemic clamp studies were performed exactly as described (13).

Mouse islet isolation and insulin secretion assay. Mouse pancreatic islets, isolated from 6-h fasted male PPARα$^{+/+}$ and PPARα$^{-/-}$ *ob/ob* mice (online appendix), were counted by dithizone staining. The purity of the preparations was estimated at ~60%. Identical numbers of islet equivalents (IE; reference islet diameter = 150 μm [14]; 10 IE per well, five wells per condition, $n = 3$ independent experiments) were preincubated for 30 min in RPMI medium containing 10% newborn calf serum and 2.8 mmol/l glucose and subsequently consecutively incubated for 1 h with 2.8 and 20 mmol/l glucose with or without 3-isobutyl-1-methylxanthine as indicated. At the end of each incubation period, medium was collected and insulin measured by ELISA (Mercodia). The stimulation index was defined as the ratio of stimulated over basal insulin secretion.

Islet morphology. Each pancreas was embedded in paraffin and sectioned (6 μm) throughout its length to avoid bias caused by changes in islet distribution or cell composition. Sections were stained using the Papanicolaou method. In addition, cryostat sections (8 μm) were used for immunostaining of insulin and glucagon (online appendix). Cryosections of pancreas were probed for the presence of apoptotic cells (transferase-mediated dUTP nick-end labeling assay) using the ApopTag fluorescein in situ apoptosis detection kit (Qbiogene; MP Biomedicals, Illkirch, France).

Morphometric analysis. Pancreas sections were randomly chosen at fixed intervals (every 40th section) to ensure representation of the whole pancreas. Morphometric parameters were determined using a Leica microscope and a color video camera coupled to the Quips Image Analysis System (Leica Mikroskopic und System, Wetzlar, Germany). Measurement of the area of pancreatic islets, as well as that of total pancreatic sections, was manually performed using Quora's tablet work surface coupled to the same computerized system.

RNA extraction and quantitative PCR analysis. RNA, isolated from the pancreas using the acid guanidium thiocyanate/phenol/chloroform method (15), was reverse transcribed using Moloney murine leukemia virus–reverse transcriptase (Invitrogen, France) and random hexamer primers. mRNA levels of the indicated genes were quantified by real-time quantitative PCR on a Mx-4000 apparatus (Stratagene) using specific primers (online appendix).

Pancreas insulin content. Pancreas insulin content was measured by ELISA (Mercodia) after insulin extraction with acidic ethanol (0.2 mol/l HCl in 75% ethanol) and normalized to protein content.

Human tissues and culture conditions. Human pancreata were harvested from brain-dead nondiabetic adult donors (age 46 ± 4 years, BMI 26.8 ± 2.7 kg/m^2, HbA_{1c} 5.12 ± 0.15%, $n = 3$), in agreement with French regulations and with the local institutional ethical committee, and pancreatic islets were isolated and purified (online appendix). The islet numbers were determined on each preparation by dithizone staining and expressed as the number of islet equivalents with a 150-μm diameter (IE) (14). Preparations used in this study exhibited an 82 ± 6% purity of endocrine tissue.

Islets were incubated with BSA-bound palmitate (0.33 mmol/l, molar ratio of palmitate to BSA 6:1 [16]) and the indicated agonists for 48 h.

Single purified β-cells were isolated (17) and PPARα expression analyzed by quantitative PCR.

Acute insulin release. To determine acute insulin release in response to glucose stimulation (18), islets were preincubated for 30 min in RPMI medium (Sigma Aldrich) containing 10% newborn calf serum and 2.8 mmol/l glucose and subjected to two successive 1-h incubations with 2.8 (basal) and 20 (stimulation) mmol/l glucose. At the end of each incubation period, medium was collected and insulin measured using a bi-insulin immunoradiometric assay kit (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). The stimulation index was defined as the ratio of stimulated over basal insulin secretion.

Islet triglyceride content. Islet preparations were sonicated in 0.9% NaCl and triglycerides measured using the Trinder method (Triglycerides GPO-PAP kit; Roche Diagnostics).

DNA fragmentation assay. DNA fragmentation was assessed in the cytoplasmic fractions of islets using the Cell Death Detection ELISA kit from Roche Molecular Biochemicals (Mannheim, Germany).

Statistical analysis. Results are reported as the means ± SE. Data were compared using Student's t test for two-group comparison or ANOVA for multigroup comparison. Significant differences were post hoc analyzed using the Scheffe test. A value of $P < 0.05$ was considered significant.

RESULTS

PPARα deficiency does not affect body weight gain of *ob/ob* mice. Body weight evolution from 4 until 20 weeks of age was evaluated in obese *ob/ob* PPARα$^{+/+}$ and PPARα$^{-/-}$ mice and in control lean *OB/OB* littermates (Fig. 1*A* and *B*). As expected, from week 4 on, *ob/ob* mice gained weight rapidly in comparison with *OB/OB* mice. PPARα deficiency did not influence the growth curves of either *OB/OB* or *ob/ob* male or female mice (Fig. 1*A* and *B*).

PPARα deficiency aggravates the age-dependent onset of hyperglycemia in *ob/ob* mice. Blood glucose concentrations were measured in the same mice after a 6-h fasting period (Fig. 1*C* and *D*). In agreement with previous observations (11,19), compared with *OB/OB* mice, blood glucose concentrations were lower in PPARα-deficient *OB/OB* male mice at all ages (6 weeks 155 ± 7 mg/dl in PPARα$^{+/+}$ *OB/OB*, 130 ± 5 mg/dl in PPARα$^{-/-}$ *OB/OB*, $P < 0.01$; 16 weeks 146 ± 6 mg/dl in PPARα$^{+/+}$ *OB/OB*, 116 ± 6 mg/dl in PPARα$^{-/-}$ *OB/OB*, $P < 0.05$). Obese *ob/ob* mice exhibited an increase in plasma glucose concentrations already from 6 weeks of age on (6 weeks 206 ± 12 mg/dl in PPARα$^{+/+}$ *ob/ob*), stabilizing thereafter at moderately elevated levels (16 weeks 174 ± 11 mg/dl in PPARα$^{+/+}$ *ob/ob*) (Fig. 1*C*). Surprisingly, PPARα deficiency in the obese background resulted in a more pronounced hyperglycemia (Fig. 1*C*). This hyperglycemia appeared already within 6 weeks of age (231 ± 17 mg/dl in male PPARα$^{-/-}$ *ob/ob*) and became maximal from the age of 10–12 weeks on (16 weeks 336 ± 28 mg/dl in male PPARα$^{-/-}$ *ob/ob*). Similar, albeit less pronounced, changes in plasma glucose were observed in female mice (Fig. 1*D*). Thus, in the obese background, PPARα deficiency aggravates the onset of metabolic perturbations of glucose homeostasis. Similar to previous observations (20,21), the phenotype was most pronounced in male mice. Further studies were thus performed in male mice only.

PPARα deficiency in *ob/ob* mice results in metabolic alterations of glucose homeostasis. To explore the origin of the metabolic perturbation of glucose homeosta-

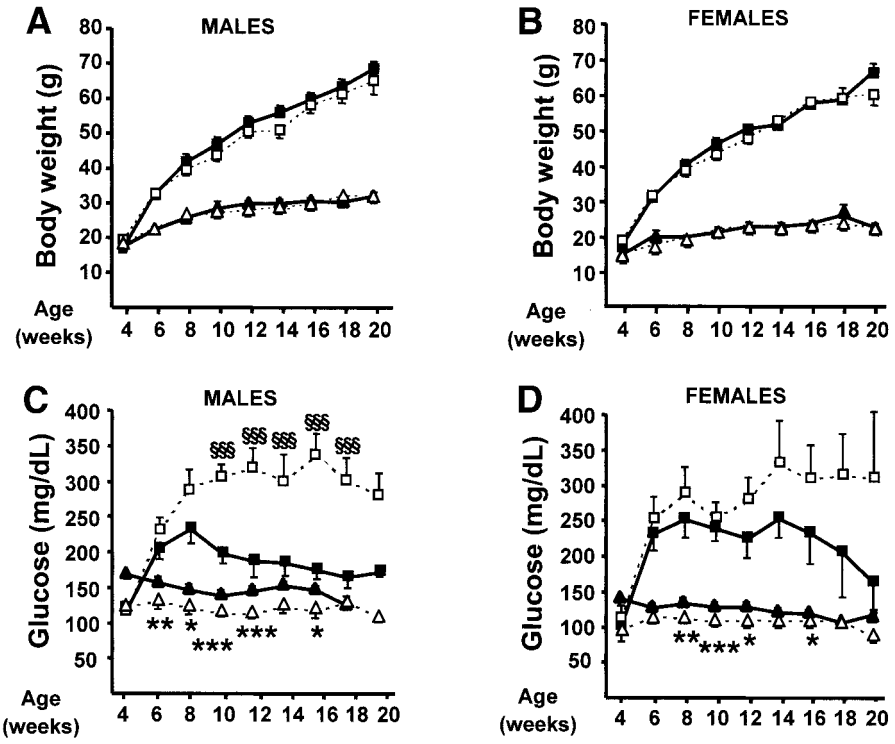

FIG. 1. PPARα deficiency does not influence body weight gain but aggravates hyperglycemia with age in *ob/ob* mice. Body weights (*A* and *B*) and tail vein blood glucose levels obtained after a 6-h fast (*C* and *D*) were measured in PPARα$^{+/+}$ (■) and PPARα$^{-/-}$ (□) *ob/ob* mice and in PPARα$^{+/+}$ (▲) and PPARα$^{-/-}$ (△) *OB/OB* mice, in both males (*A* and *C*) and females (*B* and *D*) (n = 10/genotype). Results are expressed as means ± SE. Statistical differences are indicated by § between *ob/ob* mice (§§§P < 0.001) and by * between *OB/OB* mice (*P < 0.05, **P < 0.01, and ***P < 0.001).

sis in PPARα-deficient obese mice, the phenotype was further analyzed in 13- to 16-week-old male mice of each genotype, an age at which they exhibit a stable phenotype. As in Fig. 1*C*, PPARα$^{-/-}$ *ob/ob* mice developed a more pronounced hyperglycemia than PPARα$^{+/+}$ *ob/ob* mice (Fig. 2*A*).

As previously reported (22), PPARα$^{+/+}$ *ob/ob* mice displayed a marked hyperinsulinemia (6.2 ± 1.9 μg/l) when compared with control *OB/OB* mice (0.7 ± 0.1 μg/l), due to an increase in insulin secretion by the pancreas to compensate for the peripheral insulin resistance (Fig. 2*B*). Surprisingly, notwithstanding large variations in this parameter, PPARα$^{-/-}$ *ob/ob* mice displayed lower plasma insulin concentrations (3.5 ± 1.9 μg/l) than PPARα$^{+/+}$ *ob/ob* mice (6.2 ± 1.9 μg/l), pointing to a potential defective compensatory secretion of insulin by the pancreatic β-cells of PPARα$^{-/-}$ *ob/ob* mice. This hypothesis was supported by the calculation of the ratio of glucose (mg/dl) to insulin (μg/l), which was significantly increased in PPARα$^{-/-}$ *ob/ob* mice as compared with PPARα$^{+/+}$ *ob/ob* mice (194 ± 40 in PPARα$^{-/-}$ *ob/ob* mice vs. 70 ± 40 in PPARα$^{+/+}$ *ob/ob* mice, $P < 0.05$). In contrast to *ob/ob* mice, insulin levels in PPARα$^{-/-}$ *OB/OB* mice tended to be higher (1.27 ± 0.29 μg/l) than in PPARα$^{+/+}$ *OB/OB* mice (0.69 ± 0.10 μg/l) (Fig. 2*B*).

Plasma FFAs were increased to a similar extent in PPARα$^{-/-}$ *ob/ob* mice and in PPARα$^{-/-}$ *OB/OB* mice, suggesting a similar impact of PPARα deficiency on the peripheral-hepatic axis of fatty acid transport and metabolism in obese and nonobese mice (Fig. 2*C*).

PPARα deficiency does not aggravate peripheral insulin resistance in *ob/ob* mice. Glucose tolerance tests and hyperinsulinemic-euglycemic clamps were performed to evaluate whether PPARα deficiency aggravates peripheral insulin resistance in *ob/ob* mice. After IPGTTs (Fig. 3*A*) or OGTTs (Fig. 3*B*), the dynamic glucose excursion curves revealed no significant increase in PPARα$^{-/-}$ *ob/ob* mice as compared with PPARα$^{+/+}$ *ob/ob* mice, as evi-

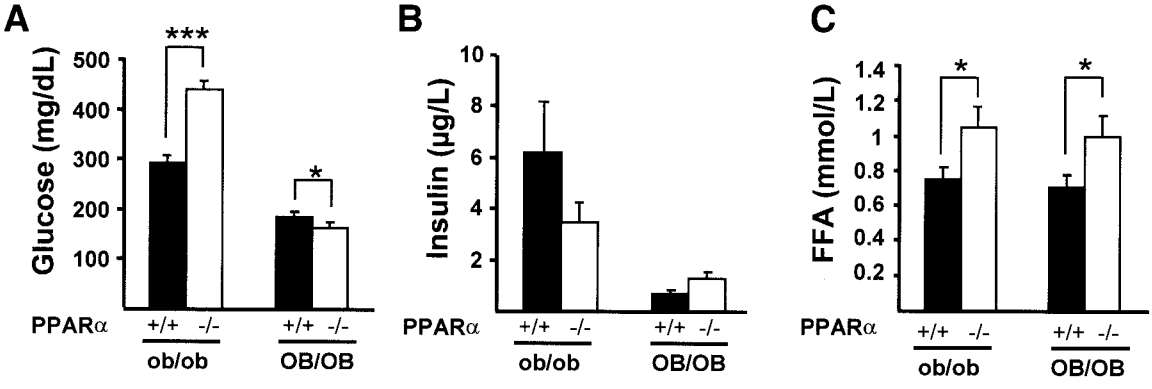

FIG. 2. PPARα deficiency alters glucose homeostasis in *ob/ob* mice. Glucose (*A*), insulin (*B*), and FFAs (*C*) were measured in blood obtained from the retro-orbital sinus of 13- to 16-week-old *ob/ob* and *OB/OB* male mice, deficient (−/−) or not (+/+) for PPARα (n = 10/genotype), which had been food deprived for 6 h. Results are expressed as means ± SE. Statistical differences are indicated between PPARα$^{+/+}$ and PPARα$^{-/-}$ mice (*P < 0.05 and ***P < 0.001).

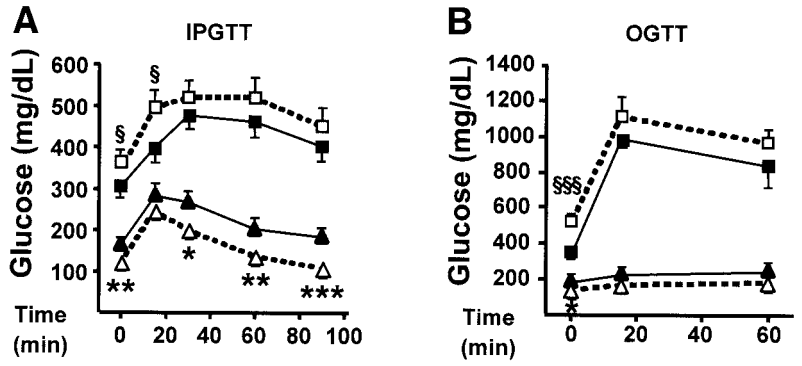

FIG. 3. PPARα deficiency does not modify the dynamic glucose excursion curves in response to a glucose load in *ob/ob* mice. *A*: IPGTTs (1 g/kg) were performed in 6-h fasted mice (n = 6–7 mice/group). Glucose levels were measured at 0, 15, 30, 60, and 90 min. Data are expressed as the means ± SE. ■, PPARα$^{+/+}$ *ob/ob* mice; □, PPARα$^{-/-}$ *ob/ob* mice; ▲, PPARα$^{+/+}$ *OB/OB* mice; △, PPARα$^{-/-}$ *OB/OB* mice. Statistical significant differences are indicated by § (§P < 0.05 and §§§P < 0.001) between *ob/ob* mice and by * between *OB/OB* mice (*P < 0.05, **P < 0.01, and ***P < 0.001) *B*: OGTTs were performed on 6-h fasted mice (n = 6–7 mice/group). Glucose levels were measured at 0, 15, and 60 min. Plasma glucose levels are expressed as means ± SE.

denced by the identical areas under the curve (IPGTT 11,410 ± 1,129 mg · dl^{-1} · min^{-1} in PPARα$^{+/+}$ *ob/ob* vs. 14,106 ± 1,747 mg · dl^{-1} · min^{-1} in PPARα$^{-/-}$ *ob/ob*, NS; OGTT 29,949 ± 1,950 mg · dl^{-1} · min^{-1} in PPARα$^{+/+}$ *ob/ob* vs. 26,813 ± 3,763 mg · dl^{-1} · min^{-1} in PPARα$^{-/-}$ *ob/ob*, NS). On the contrary, plasma blood glucose excursion curves were lower in PPARα$^{-/-}$ *OB/OB* mice as compared with PPARα$^{+/+}$ *OB/OB* mice, as previously shown (11,19).

Next, hyperinsulinemic-euglycemic clamp experiments were performed in male mice of the four genotypes. During the steady-state period (3–6 h), blood glucose levels were clamped to reach virtually identical levels in all groups (Fig. 4*A*). As expected, the glucose infusion rate (GIR) required to maintain euglycemia under insulin infusion in *ob/ob* mice was about eightfold lower than in *OB/OB* mice, confirming the existence of insulin resistance in *ob/ob* mice. Interestingly, the GIR was slightly lower in PPARα-deficient mice, both on the nonobese (707 ± 44 μmol · kg^{-1} · min^{-1} in PPARα$^{+/+}$ *OB/OB* vs. 631 ± 31μmol · kg^{-1} · min^{-1} in PPARα$^{-/-}$ *OB/OB*, P < 0.05) and obese background (101 ± 10 μmol · kg^{-1} · min^{-1} in PPARα$^{+/+}$ *ob/ob* vs. 72 ± 16 μmol · kg^{-1} · min^{-1} in PPARα$^{-/-}$ *ob/ob*, P < 0.05) (Fig. 4*B*). The glucose disposal rate, however, was similar in PPARα$^{+/+}$ and PPARα$^{-/-}$ mice both on the obese and nonobese backgrounds (Fig. 4*C*), indicating that PPARα deficiency does not modulate peripheral insulin resistance. The decreased GIR in PPARα-deficient mice was associated with a slight increase in hepatic glucose production, which was observed in both the obese and nonobese backgrounds (Fig. 4*D*), an effect which thus could not explain the hyperglycemia observed in PPARα-deficient obese mice (Fig. 1*C*).

PPARα deficiency alters pancreas islet function in *ob/ob mice*. The possibility of pancreatic β-cell dysfunction in PPARα$^{-/-}$ *ob/ob* mice was assessed by measuring the insulin response to a glucose load during the OGTT test. Compared with PPARα$^{+/+}$ *ob/ob* mice, in response to a glucose load, PPARα$^{-/-}$ *ob/ob* mice exhibited a severe decrease in first-phase insulin secretory response (Fig. 5*A*), as evidenced from the large difference in plasma insulin levels between the groups at 15 min (13.53 ± 2.04 μg/l in PPARα$^{+/+}$ *ob/ob* mice vs. 5.84 ± 1.01 μg/l in PPARα$^{-/-}$ *ob/ob*, P < 0.01). Moreover, PPARα$^{-/-}$ *ob/ob* mice exhibited a slight but not significant decrease of total pancreas insulin content (−30% compared with PPARα$^{+/+}$ *ob/ob* mice) (Fig. 5*B*).

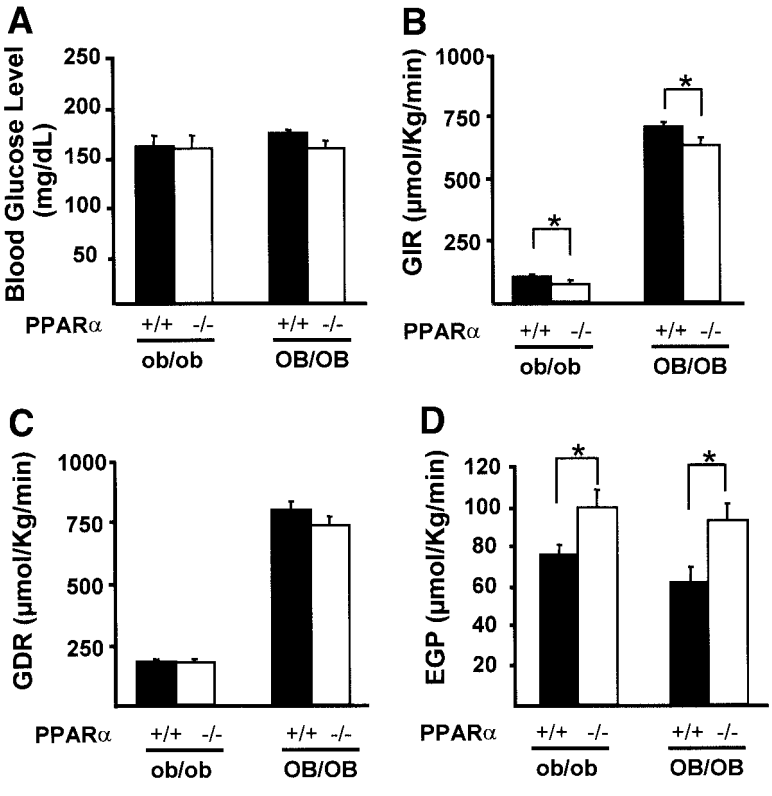

FIG. 4. PPARα deficiency does not aggravate peripheral insulin resistance in *ob/ob* mice. Clamped blood glucose levels (*A*), GIR during hyperinsulinemia (*B*), glucose disposal rates (GDRs) (*C*), and endogenous glucose production (EGP) (*D*) were measured at steady state (3–6 h) during hyperinsulinemic-euglycemic clamps performed in *ob/ob* and *OB/OB* male mice deficient (−/−) or not (+/+) for PPARα. Results are expressed as means ± SE (n = 7 mice/group). Statistical differences between PPARα$^{+/+}$ and PPARα$^{-/-}$ mice are indicated (*P < 0.05).

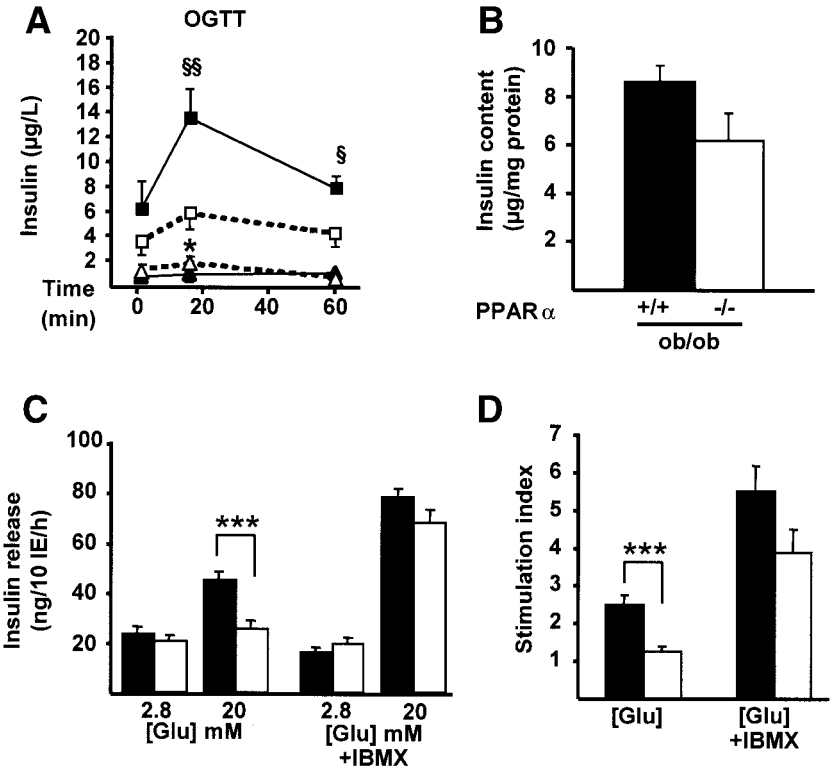

FIG. 5. PPARα deficiency impairs GSIS by islets of *ob/ob* mice. *A*: Plasma insulin levels (means ± SE) were measured at 0, 15, and 60 min during the OGTTs. Data are expressed as means ± SE. ■, PPARα$^{+/+}$ *ob/ob* mice; □, PPARα $^{-/-}$ *ob/ob* mice; ▲, PPARα$^{+/+}$ *OB/OB* mice; △, PPARα$^{-/-}$ *OB/OB* mice (n = 6–7 mice/group). Statistical differences are indicated by § (§P < 0.05 and §§P < 0.01) between *ob/ob* mice and by * between *OB/OB* mice (*P < 0.05). *B*: Pancreas insulin content was measured in PPARα$^{+/+}$ *ob/ob* (■) and PPARα$^{-/-}$ *ob/ob* (□) mice and normalized to protein content (n = 6 mice/group). *C*: Pancreas islets (10 IE/point) isolated from fasted 13- to 16-week-old male PPARα$^{+/+}$ (■) and PPARα$^{-/-}$ (□) *ob/ob* mice were incubated for two successive 1-h periods at low glucose (2.8 mmol/l) and high glucose (20 mmol/l) concentrations in the presence or absence of 3-isobutyl-1-methylxanthine (IBMX) (100 µmol/l) as indicated. Insulin release in the incubation media was measured. *D*: The stimulation index was calculated as the ratio of insulin release in high to low glucose concentrations. Each experiment on different preparations (n = 3) was performed with five wells/condition. The results are expressed as the means ± SE of the three independent experiments. Statistical differences between PPARα$^{+/+}$ and PPARα$^{-/-}$ *ob/ob* mice are indicated (*P < 0.001).**

To determine whether this decreased insulin concentration observed in vivo could be the result of a reduced insulin release per islet, GSIS was assessed in vitro in islets isolated from 6-h fasted PPARα$^{+/+}$ and PPARα$^{-/-}$ *ob/ob* mice. To appreciate qualitative alterations in islet function independent of changes in islet morphology (see below), the response of 10 identical IEs from PPARα$^{+/+}$ and PPARα$^{-/-}$ obese mice to glucose was analyzed. Whereas high glucose (20 mmol/l) stimulated insulin secretion in islets isolated from PPARα$^{+/+}$ *ob/ob* mice, islets isolated from PPARα$^{-/-}$ *ob/ob* mice displayed a total absence of GSIS (Fig. 5*C*). This strong reduction in GSIS was reflected by a 50% lower stimulation index in PPARα$^{-/-}$ *ob/ob* mice as compared with PPARα$^{+/+}$ *ob/ob* mice (Fig. 5*D*). By contrast, incubation with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine, which increases cellular cAMP through phosphodiesterase inhibition, potentiated GSIS in PPARα$^{+/+}$ *ob/ob* mice and restored insulin secretion in PPARα$^{-/-}$ *ob/ob* mice (Fig. 5*C* and *D*). Thus, PPARα deficiency in *ob/ob* mice results in impaired GSIS both in vitro and in vivo.

PPARα deficiency alters pancreas islet morphology in *ob/ob mice*. To further examine the effect of PPARα deficiency on islet and pancreas morphology, immunohistochemical studies were performed on pancreatic sections from PPARα$^{+/+}$ and PPARα$^{-/-}$ *ob/ob* mice as well as those from their lean controls. In *OB/OB* mice, PPARα deficiency did not significantly modify mean islet area (Fig. 6*A*). Since *ob/ob* mouse pancreas islets develop hyperplasia to compensate for the peripheral insulin resistance (22,23), it was assessed whether PPARα deficiency might alter this adaptative response. Interestingly, mean islet area, as a percentage of cumulative pancreatic sections, was significantly decreased (median of cumulative pancreatic sections 3.23 ± 0.44% in PPARα$^{+/+}$ *ob/ob* vs. 1.66 ± 0.41% in PPARα$^{-/-}$ *ob/ob*, P < 0.05) in PPARα$^{-/-}$ *ob/ob* mice as compared with PPARα$^{+/+}$ *ob/ob* mice (Fig. 6*A*) due to an attenuated islet hyperplasia in PPARα$^{-/-}$ *ob/ob* mice (Fig. 6*B*). Additionally, immunostaining of β-cells with insulin antibodies indicated that, although insulin staining per β-cell appeared more pronounced in PPARα$^{-/-}$ *ob/ob* mice, total islet insulin staining was decreased (−27% compared with PPARα$^{+/+}$ *ob/ob* mice), likely due to the reduced β-cell content of the islets (Fig. 6*B*). Immunostaining for glucagon revealed no quantitative differences in glucagon content or α-cell number in PPARα$^{-/-}$ *ob/ob* mice (Fig. 6*B*).

These results indicate that PPARα deficiency in *ob/ob* mice results in an alteration of pancreas morphology associated with pancreas dysfunction.

Influence of PPARα deficiency on the expression of genes in the pancreas of *ob/ob mice*. To understand how PPARα deficiency led to alterations in both pancreas function and pancreas morphology in *ob/ob* mice, gene expression analysis was performed on RNA isolated from the pancreas of 13- to 16-week-old male PPARα$^{+/+}$ and PPARα$^{-/-}$ *ob/ob* mice (Fig. 6*C*). Insulin gene expression was slightly but not significantly decreased (−30%), consistent with a nonsignificant 27% reduction in islet insulin staining. No difference was observed in glucagon gene expression, in agreement with the immunohistochemical studies. Gene expression of acyl-coA oxidase, a well-characterized PPARα target gene, was significantly lower in the pancreas of PPARα$^{-/-}$ *ob/ob* mice as compared with PPARα$^{+/+}$ *ob/ob* mice. Interestingly, a significant decrease in the expression of the gastrointestinal polypeptide receptor (GIPR) was observed in PPARα$^{-/-}$ *ob/ob* mice, whereas glucagon-like peptide-1 (GLP1) receptor and pancreatic duodenal homeobox-1 (PDX-1) mRNA levels tended to decrease, but the changes did not reach significance.

PPARα agonists increase the stimulation index in lipotoxic human pancreatic islets. To assess the role of PPARα in the human pancreas, islets were isolated by Liberase digestion and density gradient purification from

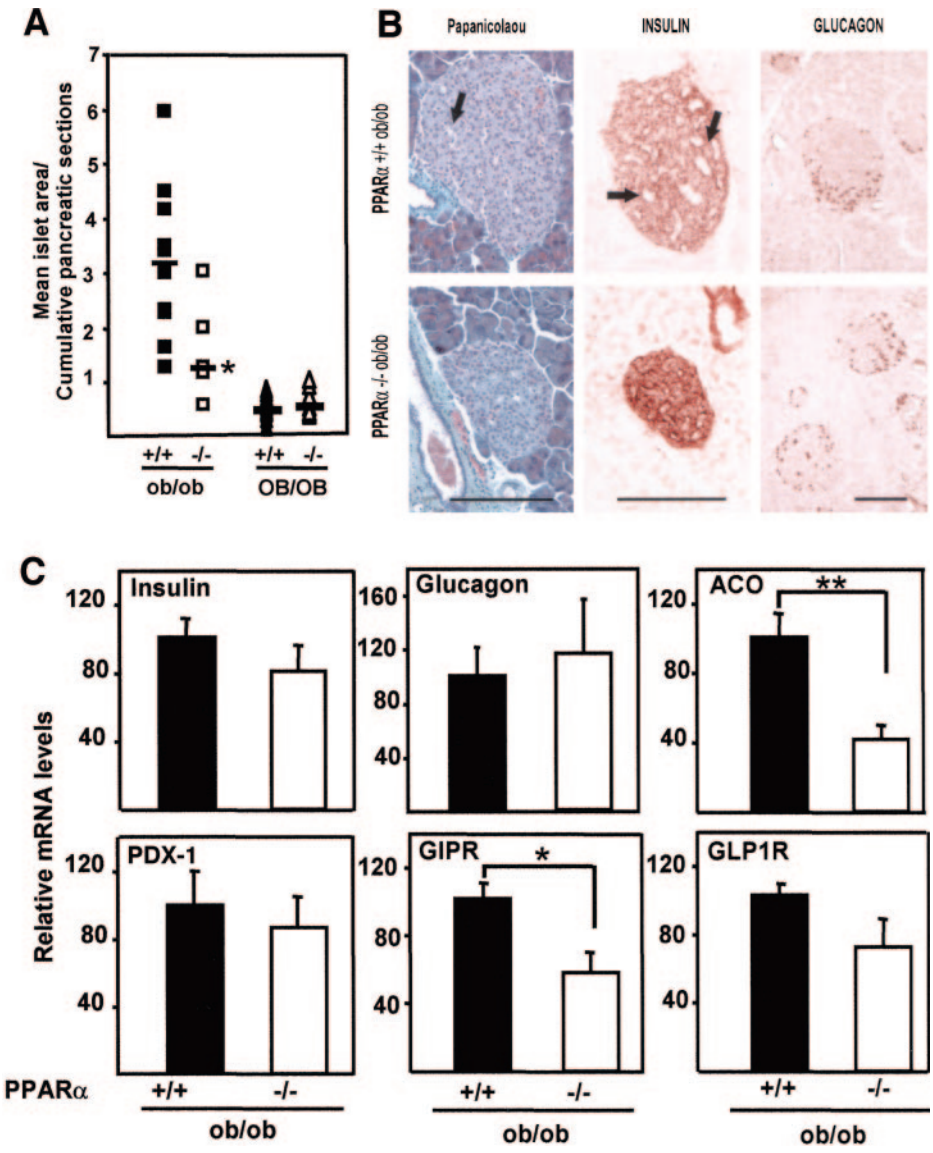

FIG. 6. PPARα deficiency alters the pancreatic phenotype of *ob/ob* mice. *A*: Mean islet areas relative to cumulative pancreatic sections were calculated for pancreases of 13- to 16-week-old mice. ■, PPARα$^{+/+}$ *ob/ob* mice (n = 10); □, PPARα$^{-/-}$ *ob/ob* mice (n = 5); ▲, PPARα$^{+/+}$ *OB/OB* mice (n = 9); △, PPARα$^{-/-}$ *OB/OB* mice (n = 8). Statistical differences between PPARα$^{+/+}$ *ob/ob* and PPARα$^{-/-}$ *ob/ob* mice are indicated (*P < 0.05). *B*: Immunohistochemical analysis of pancreas sections from PPARα$^{+/+}$ and PPARα$^{-/-}$ *ob/ob* mice stained by the Papanicolaou method (*left panels*) or with antibodies directed against insulin (*middle panels*) or glucagon (*right panels*). Bar = 200 μm. Arrows indicate the presence of capillaries in the islet. *C*: Expression of insulin, glucagon, acyl-coA oxidase (ACO), PDX-1, GIPR, and GLP1 receptor mRNA was measured by quantitative PCR in the pancreas of 13- to 16-week-old PPARα$^{+/+}$ (■) and PPARα$^{-/-}$ (□) *ob/ob* mice. RNA levels normalized to 28S RNA of PPARα$^{+/+}$ *ob/ob* mice were arbitrarily set at 100%. Results are means ± SE of five mice for each genotype (*P < 0.05 and **P < 0.01).

the pancreas of three donors. In human fluorescence-activated cell sorter–purified β-cells, PPARα RNA levels, as determined by quantitative PCR analysis, were slightly lower than in primary human hepatocytes and higher than in human aortic smooth muscle cells, two cell types known to express functional PPARα (primary human hepatocytes 100 ± 22%, primary human β-cells 65 ± 15%, and primary human aortic mooth muscle cells 37 ± 2%). The effect of synthetic PPARα activators on insulin secretion was studied in islets cultured for 48 h in CMRL-1066 medium supplemented with 0.33 mmol/l palmitate (mimicking conditions of lipotoxicity). Interestingly, both PPARα agonists (at concentrations within the range of their half-maximal effective concentration for human PPARα) as well as the PPARγ agonist rosiglitazone significantly improved the stimulation index (Fig. 7*C*). The PPARα agonists mainly acted by reducing basal insulin secretion, whereas the PPARγ agonist modulated both basal and stimulated insulin secretion (Fig. 7*A* and *B*).

As previously reported in human islets (24,25), incubation with fatty acids for 48 h resulted in triglyceride accumulation (P < 0.001) and apoptosis of islets (P < 0.001) (Fig. 7*D* and *E*). Interestingly, coincubation with the different PPARα agonists or the PPARγ agonist limited palmitate-induced lipid accumulation (Fig. 7*D*) and apoptosis (Fig. 7*E*). Moreover, the stimulation index of the islets was inversely correlated with their triglyceride content (r = 0.59; P < 0.02) and apoptosis (r = 0.64; P < 0.009). Thus, activation of PPARα reduces the vulnerability of human islets to palmitate-induced lipotoxicity in vitro.

DISCUSSION

The aim of our study was to investigate the role of PPARα in modulating glucose homeostasis under pathological conditions of insulin resistance in vivo in the *ob/ob* mouse and of palmitate-induced lipotoxicity in vitro in isolated human pancreas islets.

First, our results demonstrate that PPARα deficiency in leptin-deficient obese mice does not modify weight gain but aggravates the development of hyperglycemia with age. The effect of PPARα deficiency in the obese background is not due to a deterioration of peripheral insulin resistance, as shown by hyperinsulinemic-euglycemic clamps and glucose tolerance tests. PPARα deficiency rather results in a defective compensatory insulin secretion by the β-cells. This pancreas dysfunction is supported

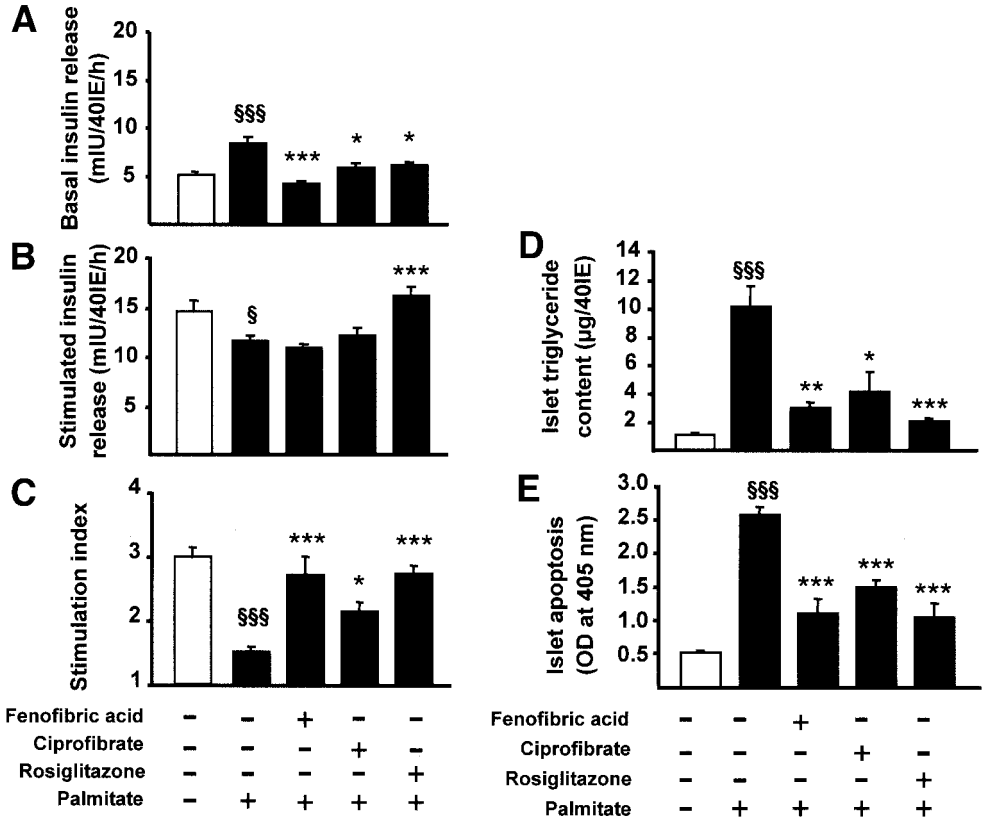

FIG. 7. PPARα agonists increase GSIS and decrease triglyceride accumulation and apoptosis in lipotoxic human pancreatic islets. Human pancreatic islets (n = 3 donors) were cultured for 48 h without (□) or with (0.33 mmol/l; ■) palmitate in the presence of the PPARα agonist fenofibric acid (50 µmol/l), ciprofibrate (50 µmol/l), or the PPARγ agonist rosiglitazone (1 µmol/l). Insulin release was measured after successive incubations in 2.8 mmol/l (basal insulin release) (*A*) and in 20 mmol/l (stimulated insulin release) (*B*). The stimulation index (*C*) was calculated as the fold increase in insulin release measured in 20 over 2.8 mmol/l glucose. Experiments were performed on islets of three different donors, using five wells/point and 40 IE/well. Results are expressed as the means ± SE of three different donors (§P < 0.05 and §§§P < 0.001 versus control; *P < 0.05 and ***P < 0.001 versus palmitate). Islet triglyceride content (*D*) and apoptosis (*E*) were measured in duplicate, as described in RESEARCH DESIGN AND METHODS. Results are expressed as the means ± SE of the three different donors, using 500 IE/well (§§§P < 0.001 versus control; *P < 0.05, **P < 0.01, and ***P < 0.001 versus palmitate).

by several findings: *1*) decreased insulin immunostaining in total pancreatic sections due to a reduction in mean islet area, *2*) decreased insulin expression in the pancreas, and *3*) decreased insulin response to glucose in vitro and in vivo. Thus, PPARα appears essential for proper adaptation of the endocrine pancreas to conditions of severe obesity-related insulin resistance associated with the absence of leptin.

A role of PPARα in modulating islet function has been suggested by the demonstration that PPARα is expressed in rat pancreatic islets, in purified rat β-cells, and in the INS-1 and HIT-T15 insulinoma cell lines (26–29). Under normal diet, insulin secretion from freshly isolated islets from wild-type or PPARα-deficient C57BL/6 mice is similar (11). Interestingly, a role for PPARα in the adaptation of pancreatic islets to fasting was recently highlighted by Gremlich et al. (19), who showed that in conditions of low glucose and fasting, PPARα deficiency results in a decrease of islet fatty acid β-oxidation activity and an impairment of fasting-induced suppression of insulin secretion in mice. These consequences of PPARα deficiency likely contribute to the hypoglycemia observed in fasted PPARα$^{-/-}$ *OB/OB* mice and the slight decrease in glucose excursion curves during glucose tolerance tests. Upon high-fat diet feeding, islets from PPARα-deficient mice present unaltered GSIS as compared with wild-type mice (11). However, PPARα-deficient mice are protected from the development of diet-induced insulin resistance (11). We therefore speculate that the preserved peripheral insulin sensitivity in PPARα-deficient mice on a nonsusceptible genetic background may protect the mice from developing pancreas dysfunction.

In several insulin-resistant rodent models, administration of PPARα agonists improved β-cell function. Fenofibrate treatment of OLETF rats prevented the development of diabetes by improving islet morphology and β-cell mass (8). In vivo treatment of high-fat–fed rats with the PPARα agonist WY14643 reversed insulin hypersecretion induced by high-fat feeding in isolated perifused islets (10). However, improvement of β-cell function under these experimental conditions of PPARα agonist treatment was associated with an improvement of peripheral insulin action, and the response of the pancreas could thus be the reflection of enhanced whole-body insulin sensitivity, precluding definitive conclusions on the role of PPARα in pancreas function. By contrast, in our study, peripheral insulin resistance is not altered in the PPARα$^{-/-}$ *ob/ob* mice, suggesting that the aggravation of hyperglycemia in these mice is due to a lack of an appropriate compensatory response of the pancreas.

At present, the mechanism behind the pancreas dysfunction due to PPARα deficiency in leptin-deficient mice is not totally defined, but it does not seem to involve an apoptotic pathway (data not shown). Since PPARα is a transcription factor, the expression of several genes controlling β-cell function was analyzed. mRNA levels of acyl-coA oxidase, a well-characterized PPARα target gene (30), were decreased in PPARα$^{-/-}$ *ob/ob* compared with PPARα$^{+/+}$ *ob/ob* mice, thereby confirming a role for PPARα in the regulation of pancreatic gene transcription. The expression of genes known to be implicated in pancreas development and function, such as the PDX-1 or different incretin receptors (the receptor of the glucagon-like peptide-1 [GLP1] and the GIPR), was also measured. The expression of these genes was increased in the pancreas of *ob/ob* compared with *OB/OB* mice (data not shown). Interestingly, whereas mRNA levels of GLP1 receptor and PDX-1 only tended to decrease, a significant decrease in GIPR gene expression was observed in PPARα$^{-/-}$ *ob/ob* mice compared with PPARα$^{+/+}$ *ob/ob*

mice. Our results extend data from a recent report showing direct regulation of GIPR gene expression by PPARα in vitro in INS(832/13) cells (31). GIP is a gastrointestinal hormone whose primary role is to stimulate insulin secretion from the pancreas in concert with glucose (32). In addition to increasing insulin secretion, GIP stimulates differentiation and proliferation of β-cells (33). Although we have no functional data demonstrating dysfunction of the GIPR pathway in our model, it is tempting to speculate that the decrease in GIPR expression contributes to the lowered islet size and secretion of insulin in PPARα$^{-/-}$ *ob/ob* mice. Further studies are required to determine whether this decrease of GIPR could contribute to the pancreatic defects in PPARα$^{-/-}$ *ob/ob* mice.

In addition to these studies in mice, we analyzed a possible role of PPARα in preserving human β-cell function in vitro upon induction of lipotoxicity with palmitate. Previously, Zhou et al. (26) showed decreased PPARα expression and absence of effect of its ligand clofibrate in islets of Zucker diabetic fatty rats. Moreover, recent reports showed that variation in the PPARα gene influences age of onset and progression of type 2 diabetes (34) and that the PPARα agonist bezafibrate reduces the incidence and delays the onset of type 2 diabetes in patients with impaired fasting glucose (35). Our results demonstrate that PPARα is also expressed in purified human pancreatic β-cells. Moreover, under conditions of lipotoxicity induced by chronic fatty acid exposure (25,36), different PPARα agonists (fenofibric acid and ciprofibrate) as well as the PPARγ agonist rosiglitazone significantly improved insulin secretion and the stimulation index in primary human islets, mainly by reducing basal insulin secretion. These functional improvements were correlated with a decrease in islet triglyceride content and palmitate-induced apoptosis. Thus, PPARα agonist treatment improves β-cell function also in human islets in vitro under pathological conditions of lipotoxicity. It was recently demonstrated that PPARα activation prevented lipid accumulation by increasing fatty acid oxidation in the INS-1E rat β-cell line (37). Thus, the most likely explanation in our human model is that PPARα agonists prevent excessive intracellular accumulation of triglycerides in islets by stimulating fatty acid β-oxidation, consequently decreasing apoptosis, and improving the insulin secretory response. Our data in this clinically relevant in vitro model thus suggest a beneficial application of PPARα agonists in the prevention of type 2 diabetes.

In conclusion, our results identify a beneficial role for PPARα in the control of pancreas function and let emerge potentially interesting therapeutic prospects for the use of PPARα agonists or PPARα/γ coagonists in the prevention of type 2 diabetes.

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