

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. PPAR α -deficient ($-/-$) mice are protected from high-fat diet–induced insulin resistance. However, the impact of PPAR α in the pathophysiological setting of obesity-related insulin resistance is unknown. Therefore, PPAR $\alpha^{-/-}$ mice in an obese (*ob/ob*) background were generated. PPAR α deficiency did not influence the growth curves of the obese mice but surprisingly resulted in a severe, age-dependent hyperglycemia. PPAR α deficiency did not aggravate peripheral insulin resistance. By contrast, PPAR $\alpha^{-/-}$ *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, PPAR α agonist treatment prevented fatty acid–induced impairment of glucose-stimulated insulin secretion, apoptosis, and triglyceride accumulation. These results indicate that PPAR α improves the adaptative response of the pancreatic β -cell to pathological conditions. PPAR α could thus represent a promising target in the prevention of type 2 diabetes. *Diabetes* 55:1605–1613, 2006

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. PPAR α 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. PPAR α regulates the expression of genes involved in fatty acid and lipoprotein metabolism (1). PPAR α 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, PPAR α -deficient mice develop a more severe hypoglycemia upon fasting, indicating a role for PPAR α in glucose metabolism as well (2–4).

Studies performed in rodent models of insulin resistance showed that PPAR α 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). PPAR α is expressed in rat pancreatic islets, and PPAR α agonist treatment has been reported to improve pancreatic β -cell function in insulin-resistant rodents (8,10). Surprisingly, PPAR α -deficient mice are protected from diet-induced insulin resistance (11). Moreover, isolated islets from normal diet– and high-fat–fed wild-type or PPAR α -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 PPAR α in the pancreas.

Since the impact of PPAR α deficiency on glucose homeostasis in the context of obesity-related insulin resistance has not yet been established, we first analyzed the consequences of PPAR α deficiency in a genetic model of obesity-related insulin resistance. PPAR α -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

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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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to produce leptin (12). Our results show that PPAR α 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 PPAR α activation was also investigated in human islets under conditions of high palmitate exposure. Our results show that PPAR α agonist treatment reduces triglyceride accumulation and apoptosis and increases the efficacy of glucose to induce insulin release. Altogether, these data indicate that PPAR α 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 (PPAR α ^{-/-} *ob/ob*) or not for PPAR α (PPAR α ^{+/+} *ob/ob*) were generated (online appendix [available at <http://diabetes.diabetesjournals.org>]). Their phenotypes were compared with those of nonobese mice deficient (PPAR α ^{-/-} *OB/OB*) or not (PPAR α ^{+/+} *OB/OB*) for PPAR α .

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, 1,500g, 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 PPAR α ^{+/+} and PPAR α ^{-/-} *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 μ m [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 μ m) 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 μ m) 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 Mikroskopische 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 \pm 4 years, BMI 26.8 \pm 2.7

kg/m², HbA_{1c} 5.12 \pm 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- μ m diameter (IE) (14). Preparations used in this study exhibited an 82 \pm 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 PPAR α 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 \pm 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

PPAR α 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* PPAR α ^{+/+} and PPAR α ^{-/-} mice and in control lean *OB/OB* littermates (Fig. 1A and B). As expected, from week 4 on, *ob/ob* mice gained weight rapidly in comparison with *OB/OB* mice. PPAR α deficiency did not influence the growth curves of either *OB/OB* or *ob/ob* male or female mice (Fig. 1A and B). **PPAR α 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. 1C and D). In agreement with previous observations (11,19), compared with *OB/OB* mice, blood glucose concentrations were lower in PPAR α -deficient *OB/OB* male mice at all ages (6 weeks 155 \pm 7 mg/dl in PPAR α ^{+/+} *OB/OB*, 130 \pm 5 mg/dl in PPAR α ^{-/-} *OB/OB*, $P < 0.01$; 16 weeks 146 \pm 6 mg/dl in PPAR α ^{+/+} *OB/OB*, 116 \pm 6 mg/dl in PPAR α ^{-/-} *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 \pm 12 mg/dl in PPAR α ^{+/+} *ob/ob*), stabilizing thereafter at moderately elevated levels (16 weeks 174 \pm 11 mg/dl in PPAR α ^{+/+} *ob/ob*) (Fig. 1C). Surprisingly, PPAR α deficiency in the obese background resulted in a more pronounced hyperglycemia (Fig. 1C). This hyperglycemia appeared already within 6 weeks of age (231 \pm 17 mg/dl in male PPAR α ^{-/-} *ob/ob*) and became maximal from the age of 10–12 weeks on (16 weeks 336 \pm 28 mg/dl in male PPAR α ^{-/-} *ob/ob*). Similar, albeit less pronounced, changes in plasma glucose were observed in female mice (Fig. 1D). Thus, in the obese background, PPAR α 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.

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

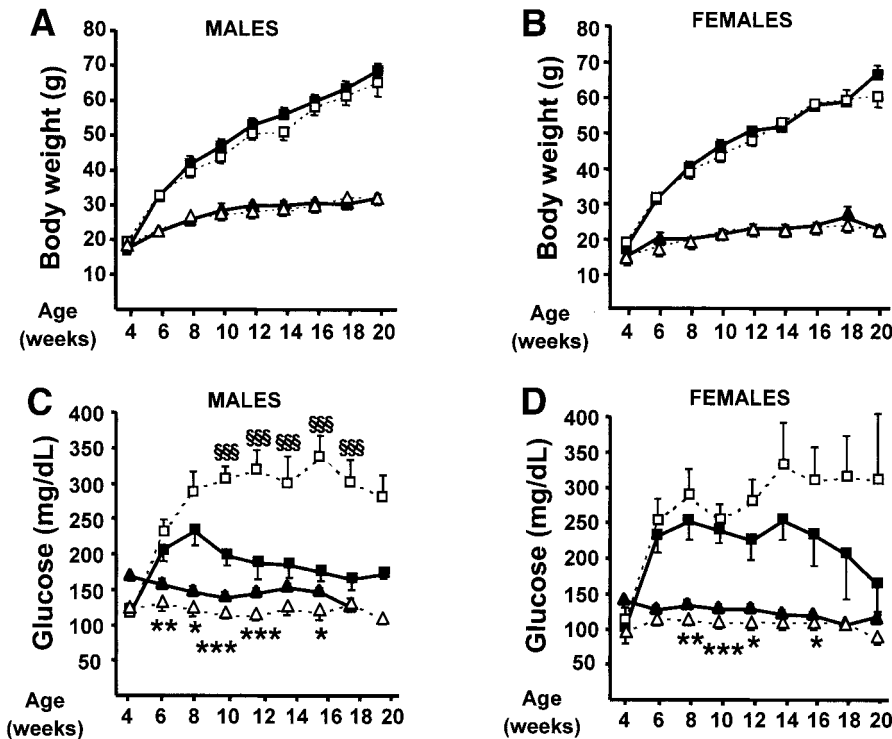


FIG. 1. PPAR α deficiency does not influence body weight gain but aggravates hyperglycemia with age in *ob/ob* mice. Body weights (A and B) and tail vein blood glucose levels obtained after a 6-h fast (C and D) were measured in PPAR $\alpha^{+/+}$ (■) and PPAR $\alpha^{-/-}$ (□) *ob/ob* mice and in PPAR $\alpha^{+/+}$ (▲) and PPAR $\alpha^{-/-}$ (△) *OB/OB* mice, in both males (A and C) and females (B and D) ($n = 10$ /genotype). Results are expressed as means \pm 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 PPAR α -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. 1C, PPAR $\alpha^{-/-}$ *ob/ob* mice developed a more pronounced hyperglycemia than PPAR $\alpha^{+/+}$ *ob/ob* mice (Fig. 2A).

As previously reported (22), PPAR $\alpha^{+/+}$ *ob/ob* mice displayed a marked hyperinsulinemia ($6.2 \pm 1.9 \mu\text{g/l}$) when compared with control *OB/OB* mice ($0.7 \pm 0.1 \mu\text{g/l}$), due to an increase in insulin secretion by the pancreas to compensate for the peripheral insulin resistance (Fig. 2B). Surprisingly, notwithstanding large variations in this parameter, PPAR $\alpha^{-/-}$ *ob/ob* mice displayed lower plasma insulin concentrations ($3.5 \pm 1.9 \mu\text{g/l}$) than PPAR $\alpha^{+/+}$ *ob/ob* mice ($6.2 \pm 1.9 \mu\text{g/l}$), pointing to a potential defective compensatory secretion of insulin by the pancreatic β -cells of PPAR $\alpha^{-/-}$ *ob/ob* mice. This hypothesis was supported by the calculation of the ratio of glucose (mg/dl) to insulin ($\mu\text{g/l}$), which was significantly increased in

PPAR $\alpha^{-/-}$ *ob/ob* mice as compared with PPAR $\alpha^{+/+}$ *ob/ob* mice (194 ± 40 in PPAR $\alpha^{-/-}$ *ob/ob* mice vs. 70 ± 40 in PPAR $\alpha^{+/+}$ *ob/ob* mice, $P < 0.05$). In contrast to *ob/ob* mice, insulin levels in PPAR $\alpha^{-/-}$ *OB/OB* mice tended to be higher ($1.27 \pm 0.29 \mu\text{g/l}$) than in PPAR $\alpha^{+/+}$ *OB/OB* mice ($0.69 \pm 0.10 \mu\text{g/l}$) (Fig. 2B).

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

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

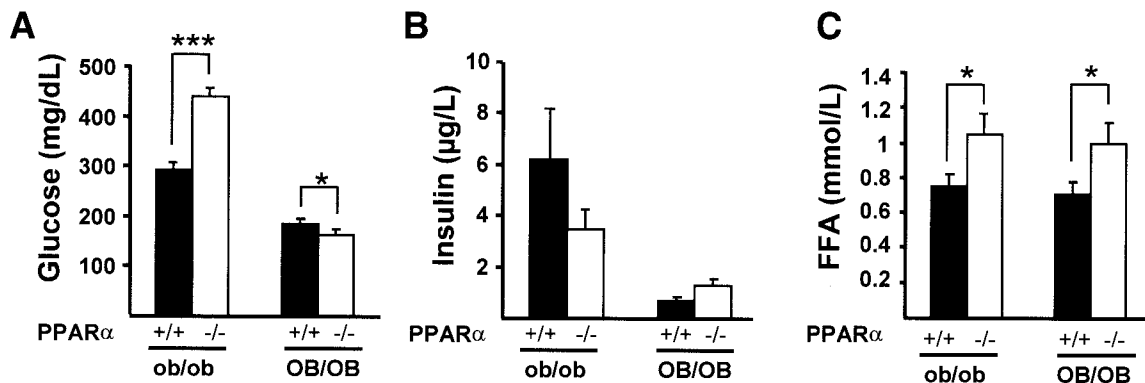


FIG. 2. PPAR α deficiency alters glucose homeostasis in *ob/ob* mice. Glucose (A), insulin (B), and FFAs (C) were measured in blood obtained from the retro-orbital sinus of 13- to 16-week-old *ob/ob* and *OB/OB* male mice, deficient (-/-) or not (+/+) for PPAR α ($n = 10$ /genotype), which had been food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

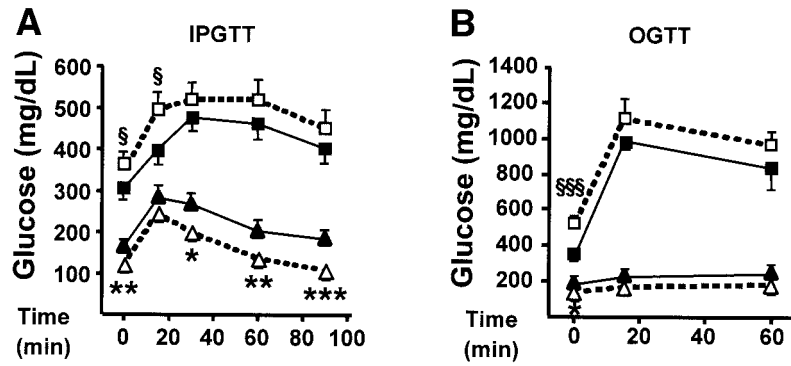


FIG. 3. PPAR α deficiency does not modify the dynamic glucose excursion curves in response to a glucose load in *ob/ob* mice. **A**: IPGTTs (1 g/kg) were performed in 6-h fasted mice ($n = 6-7$ mice/group). Glucose levels were measured at 0, 15, 30, 60, and 90 min. Data are expressed as the means \pm SE. ■, PPAR $\alpha^{+/+}$ *ob/ob* mice; □, PPAR $\alpha^{-/-}$ *ob/ob* mice; ▲, PPAR $\alpha^{+/+}$ *OB/OB* mice; △, PPAR $\alpha^{-/-}$ *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 \pm SE.

denced by the identical areas under the curve (IPGTT $11,410 \pm 1,129$ mg \cdot dl $^{-1}$ \cdot min $^{-1}$ in PPAR $\alpha^{+/+}$ *ob/ob* vs. $14,106 \pm 1,747$ mg \cdot dl $^{-1}$ \cdot min $^{-1}$ in PPAR $\alpha^{-/-}$ *ob/ob*, NS; OGTT $29,949 \pm 1,950$ mg \cdot dl $^{-1}$ \cdot min $^{-1}$ in PPAR $\alpha^{+/+}$ *ob/ob* vs. $26,813 \pm 3,763$ mg \cdot dl $^{-1}$ \cdot min $^{-1}$ in PPAR $\alpha^{-/-}$ *ob/ob*, NS). On the contrary, plasma blood glucose excursion curves were lower in PPAR $\alpha^{-/-}$ *OB/OB* mice as compared with PPAR $\alpha^{+/+}$ *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. 4A). 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 PPAR α -deficient mice, both on the nonobese (707 ± 44 μ mol \cdot kg $^{-1}$ \cdot min $^{-1}$ in PPAR $\alpha^{+/+}$ *OB/OB* vs. 631 ± 31 μ mol \cdot kg $^{-1}$ \cdot min $^{-1}$ in PPAR $\alpha^{-/-}$ *OB/OB*, $P < 0.05$) and obese background (101 ± 10 μ mol \cdot kg $^{-1}$ \cdot min $^{-1}$ in PPAR $\alpha^{+/+}$ *ob/ob* vs. 72 ± 16 μ mol \cdot kg $^{-1}$ \cdot min $^{-1}$ in PPAR $\alpha^{-/-}$ *ob/ob*, $P < 0.05$) (Fig. 4B). The glucose disposal

rate, however, was similar in PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ mice both on the obese and nonobese backgrounds (Fig. 4C), indicating that PPAR α deficiency does not modulate peripheral insulin resistance. The decreased GIR in PPAR α -deficient mice was associated with a slight increase in hepatic glucose production, which was observed in both the obese and nonobese backgrounds (Fig. 4D), an effect which thus could not explain the hyperglycemia observed in PPAR α -deficient obese mice (Fig. 1C).

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

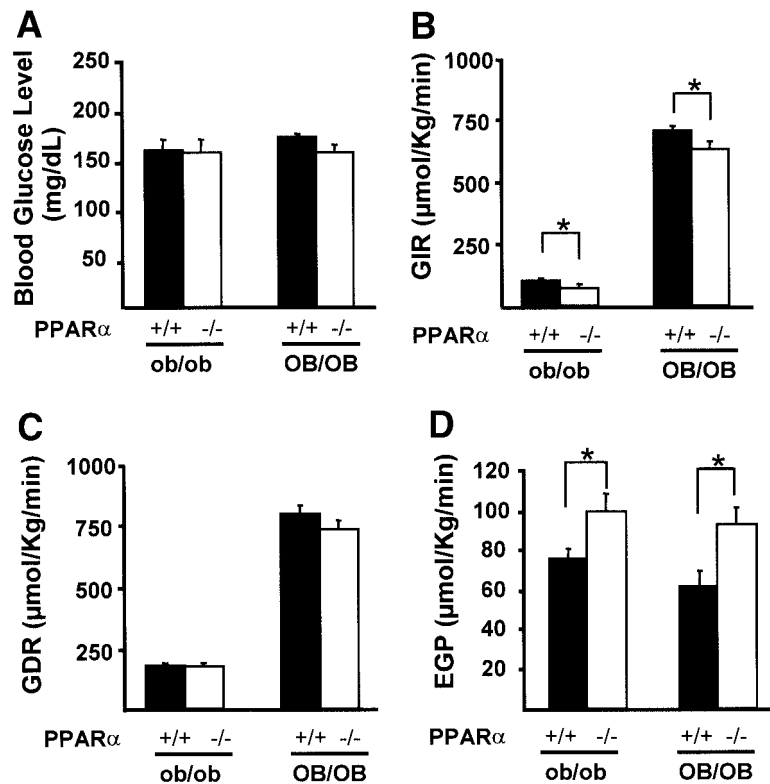


FIG. 4. PPAR α deficiency does not aggravate peripheral insulin resistance in *ob/ob* mice. Clamped blood glucose levels (**A**), GIR during hyperinsulinemia (**B**), glucose disposal rates (GDRs) (**C**), and endogenous glucose production (EGP) (**D**) were measured at steady state (3–6 h) during hyperinsulinemic-euglycemic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

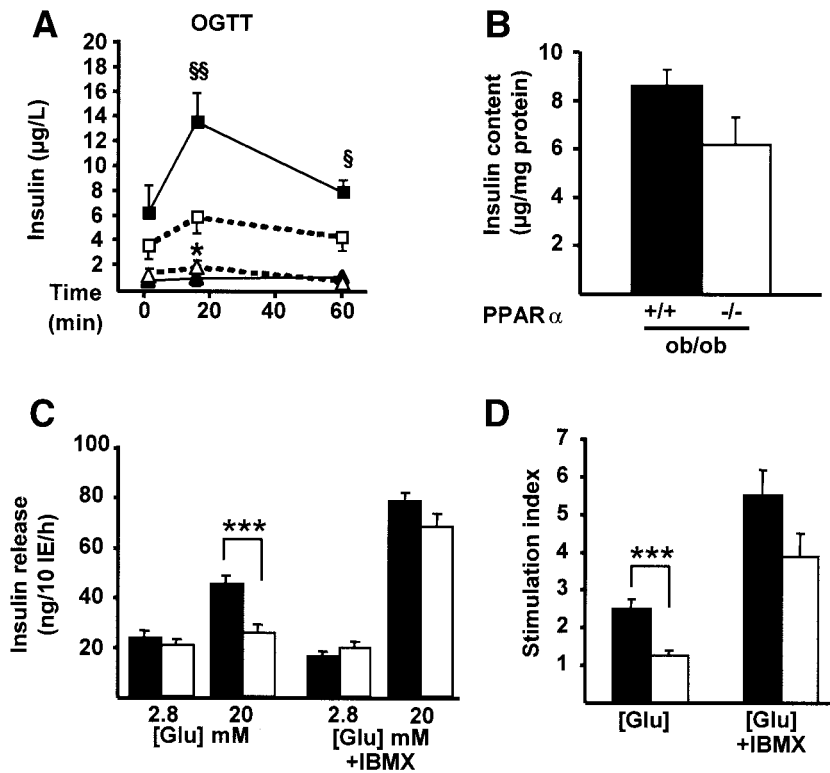


FIG. 5. PPAR α deficiency impairs GSIS by islets of *ob/ob* mice. **A:** Plasma insulin levels (means \pm SE) were measured at 0, 15, and 60 min during the OGTTs. Data are expressed as means \pm SE. ■, PPAR $\alpha^{+/+}$ *ob/ob* mice; □, PPAR $\alpha^{-/-}$ *ob/ob* mice; ▲, PPAR $\alpha^{+/+}$ *OB/OB* mice; △, PPAR $\alpha^{-/-}$ *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 PPAR $\alpha^{+/+}$ *ob/ob* (■) and PPAR $\alpha^{-/-}$ *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 PPAR $\alpha^{+/+}$ (■) and PPAR $\alpha^{-/-}$ (□) *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 μ mol/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 \pm SE of the three independent experiments. Statistical differences between PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ *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 PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ *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 PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ obese mice to glucose was analyzed. Whereas high glucose (20 mmol/l) stimulated insulin secretion in islets isolated from PPAR $\alpha^{+/+}$ *ob/ob* mice, islets isolated from PPAR $\alpha^{-/-}$ *ob/ob* mice displayed a total absence of GSIS (Fig. 5C). This strong reduction in GSIS was reflected by a 50% lower stimulation index in PPAR $\alpha^{-/-}$ *ob/ob* mice as compared with PPAR $\alpha^{+/+}$ *ob/ob* mice (Fig. 5D). By contrast, incubation with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine, which increases cellular cAMP through phosphodiesterase inhibition, potentiated GSIS in PPAR $\alpha^{+/+}$ *ob/ob* mice and restored insulin secretion in PPAR $\alpha^{-/-}$ *ob/ob* mice (Fig. 5C and D). Thus, PPAR α deficiency in *ob/ob* mice results in impaired GSIS both in vitro and in vivo.

PPAR α deficiency alters pancreas islet morphology in *ob/ob* mice. To further examine the effect of PPAR α deficiency on islet and pancreas morphology, immunohistochemical studies were performed on pancreatic sections from PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ *ob/ob* mice as well as those from their lean controls. In *OB/OB* mice, PPAR α deficiency did not significantly modify mean islet area (Fig. 6A). Since *ob/ob* mouse pancreas islets develop hyperplasia to compensate for the peripheral insulin resistance (22,23), it was assessed whether PPAR α 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 $3.23 \pm 0.44\%$ in PPAR $\alpha^{+/+}$ *ob/ob* vs. $1.66 \pm 0.41\%$ in PPAR $\alpha^{-/-}$ *ob/ob*, $P < 0.05$) in PPAR $\alpha^{-/-}$ *ob/ob* mice as compared with PPAR $\alpha^{+/+}$ *ob/ob* mice (Fig. 6A)

due to an attenuated islet hyperplasia in PPAR $\alpha^{-/-}$ *ob/ob* mice (Fig. 6B). Additionally, immunostaining of β -cells with insulin antibodies indicated that, although insulin staining per β -cell appeared more pronounced in PPAR $\alpha^{-/-}$ *ob/ob* mice, total islet insulin staining was decreased (-27% compared with PPAR $\alpha^{+/+}$ *ob/ob* mice), likely due to the reduced β -cell content of the islets (Fig. 6B). Immunostaining for glucagon revealed no quantitative differences in glucagon content or α -cell number in PPAR $\alpha^{-/-}$ *ob/ob* mice (Fig. 6B).

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

Influence of PPAR α deficiency on the expression of genes in the pancreas of *ob/ob* mice. To understand how PPAR α 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 PPAR $\alpha^{+/+}$ and PPAR $\alpha^{-/-}$ *ob/ob* mice (Fig. 6C). 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 PPAR α target gene, was significantly lower in the pancreas of PPAR $\alpha^{-/-}$ *ob/ob* mice as compared with PPAR $\alpha^{+/+}$ *ob/ob* mice. Interestingly, a significant decrease in the expression of the gastrointestinal polypeptide receptor (GIPR) was observed in PPAR $\alpha^{-/-}$ *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.

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

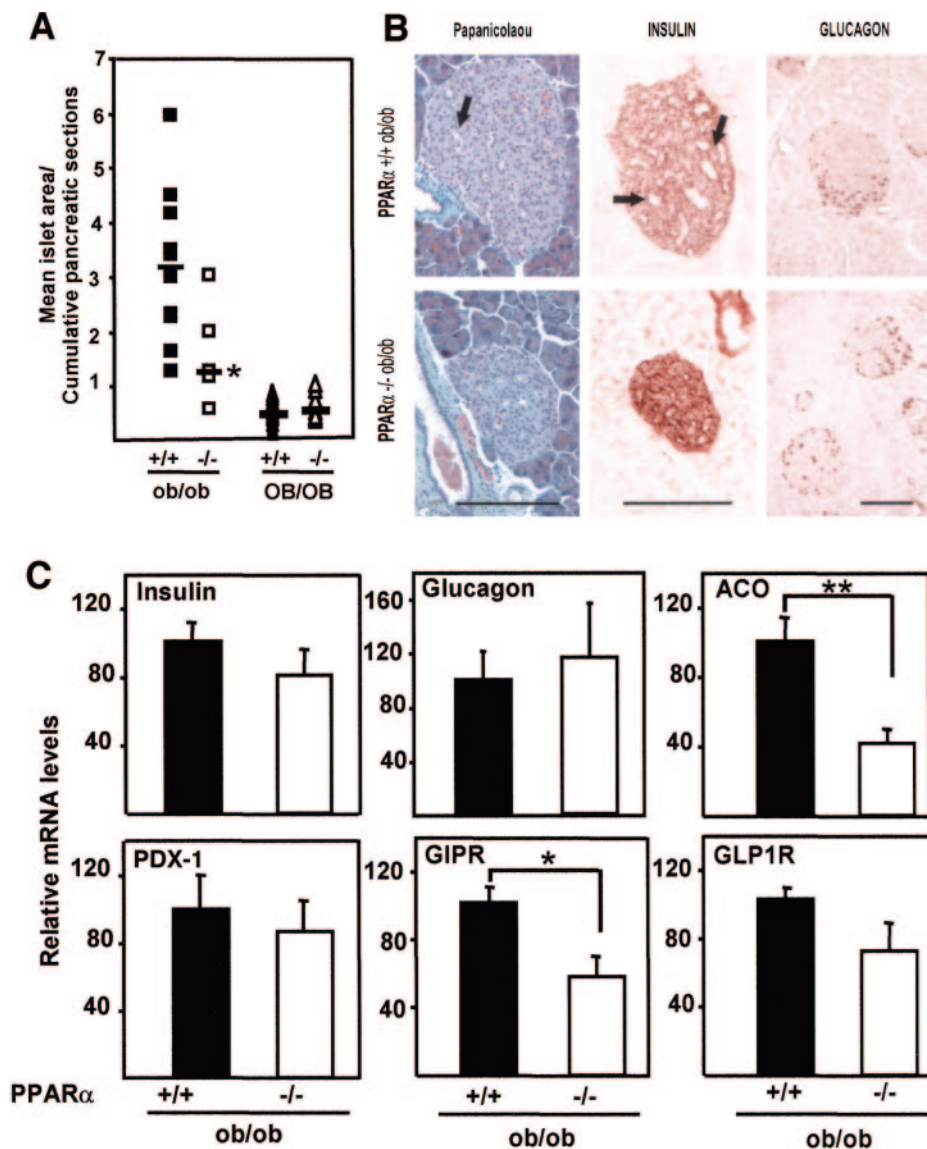


FIG. 6. PPAR α deficiency alters the pancreatic phenotype of *ob/ob* mice. **A:** Mean islet areas relative to cumulative pancreatic sections were calculated for pancreases of 13- to 16-week-old mice. ■, PPAR α ^{+/+} *ob/ob* mice ($n = 10$); □, PPAR α ^{-/-} *ob/ob* mice ($n = 5$); ▲, PPAR α ^{+/+} *OB/OB* mice ($n = 9$); △, PPAR α ^{-/-} *OB/OB* mice ($n = 8$). Statistical differences between PPAR α ^{+/+} *ob/ob* and PPAR α ^{-/-} *ob/ob* mice are indicated (* $P < 0.05$). **B:** Immunohistochemical analysis of pancreas sections from PPAR α ^{+/+} and PPAR α ^{-/-} *ob/ob* mice stained by the Papanicolaou method (left panels) or with antibodies directed against insulin (middle panels) or glucagon (right panels). Bar = 200 μ m. 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 PPAR α ^{+/+} (■) and PPAR α ^{-/-} (□) *ob/ob* mice. RNA levels normalized to 28S RNA of PPAR α ^{+/+} *ob/ob* mice were arbitrarily set at 100%. Results are means \pm 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, PPAR α 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 PPAR α (primary human hepatocytes $100 \pm 22\%$, primary human β -cells $65 \pm 15\%$, and primary human aortic smooth muscle cells $37 \pm 2\%$). The effect of synthetic PPAR α 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 PPAR α agonists (at concentrations within the range of their half-maximal effective concentration for human PPAR α) as well as the PPAR γ agonist rosiglitazone significantly improved the stimulation index (Fig. 7C). The PPAR α agonists mainly acted by reducing basal insulin secretion, whereas the PPAR γ agonist modulated both basal and stimulated insulin secretion (Fig. 7A 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. 7D and E). Interestingly, coincubation with the different PPAR α agonists or the PPAR γ agonist limited

palmitate-induced lipid accumulation (Fig. 7D) and apoptosis (Fig. 7E). 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 PPAR α reduces the vulnerability of human islets to palmitate-induced lipotoxicity in vitro.

DISCUSSION

The aim of our study was to investigate the role of PPAR α 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 PPAR α deficiency in leptin-deficient obese mice does not modify weight gain but aggravates the development of hyperglycemia with age. The effect of PPAR α 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. PPAR α deficiency rather results in a defective compensatory insulin secretion by the β -cells. This pancreas dysfunction is supported

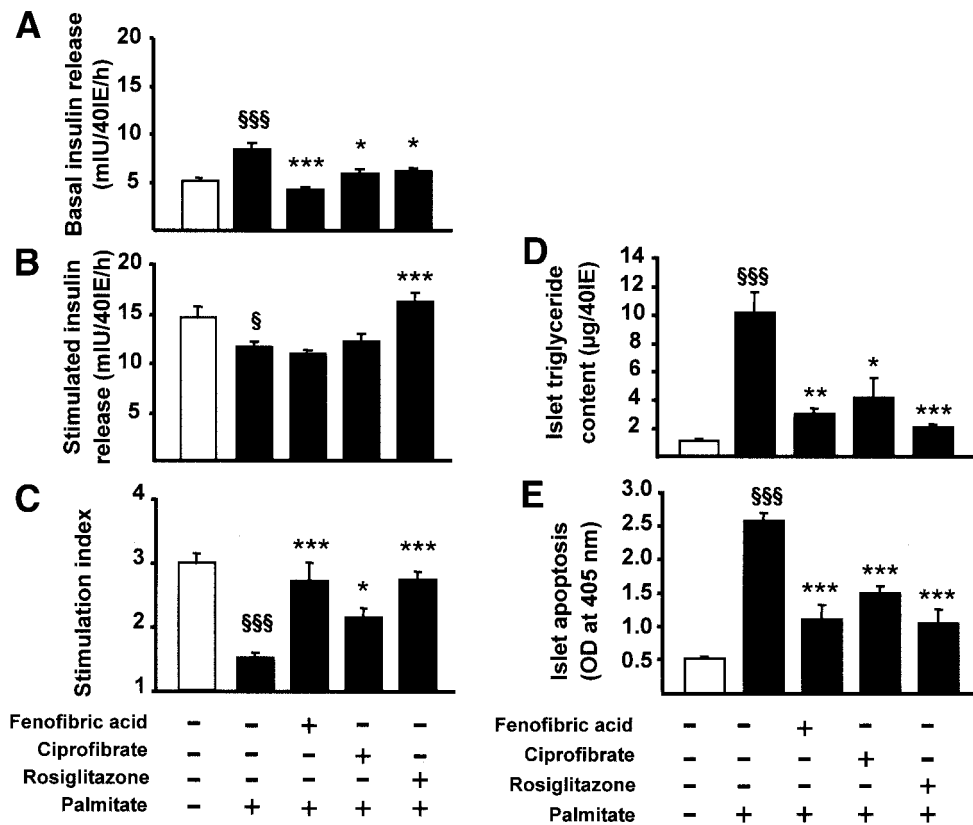


FIG. 7. PPAR α agonists increase GSIS and decrease triglyceride accumulation and apoptosis in lipotoxic human pancreatic islets. Human pancreatic islets ($n = 3$ donors) were cultured for 48 h without (\square) or with (0.33 mmol/l; \blacksquare) palmitate in the presence of the PPAR α agonist fenofibric acid (50 μ mol/l), ciprofibrate (50 μ mol/l), or the PPAR γ agonist rosiglitazone (1 μ mol/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 \pm 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 \pm 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, PPAR α 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 PPAR α in modulating islet function has been suggested by the demonstration that PPAR α 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 PPAR α -deficient C57BL/6 mice is similar (11). Interestingly, a role for PPAR α 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, PPAR α 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 PPAR α deficiency likely contribute to the hypoglycemia observed in fasted PPAR α ^{-/-} *OB/OB* mice and the slight decrease in glucose excursion curves during glucose tolerance tests. Upon high-fat diet feeding, islets from PPAR α -deficient mice present unaltered GSIS as compared with wild-type mice (11). However, PPAR α -deficient mice are protected from the development of diet-induced insulin resistance (11). We therefore speculate that the preserved peripheral insulin sensitivity in PPAR α -deficient mice on a nonsusceptible genetic background may protect the mice from developing pancreas dysfunction.

In several insulin-resistant rodent models, administration of PPAR α 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 PPAR α agonist WY14643 reversed insulin hypersecretion induced by high-fat feeding in isolated perfused islets (10). However, improvement of β -cell function under these experimental conditions of PPAR α 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 PPAR α in pancreas function. By contrast, in our study, peripheral insulin resistance is not altered in the PPAR α ^{-/-} *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 PPAR α deficiency in leptin-deficient mice is not totally defined, but it does not seem to involve an apoptotic pathway (data not shown). Since PPAR α is a transcription factor, the expression of several genes controlling β -cell function was analyzed. mRNA levels of acyl-coA oxidase, a well-characterized PPAR α target gene (30), were decreased in PPAR α ^{-/-} *ob/ob* compared with PPAR α ^{+/+} *ob/ob* mice, thereby confirming a role for PPAR α 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 PPAR α ^{-/-} *ob/ob* mice compared with PPAR α ^{+/+} *ob/ob*

mice. Our results extend data from a recent report showing direct regulation of GIPR gene expression by PPAR α 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 PPAR α ^{-/-} ob/ob mice. Further studies are required to determine whether this decrease of GIPR could contribute to the pancreatic defects in PPAR α ^{-/-} ob/ob mice.

In addition to these studies in mice, we analyzed a possible role of PPAR α in preserving human β -cell function in vitro upon induction of lipotoxicity with palmitate. Previously, Zhou et al. (26) showed decreased PPAR α expression and absence of effect of its ligand clofibrate in islets of Zucker diabetic fatty rats. Moreover, recent reports showed that variation in the PPAR α gene influences age of onset and progression of type 2 diabetes (34) and that the PPAR α agonist bezafibrate reduces the incidence and delays the onset of type 2 diabetes in patients with impaired fasting glucose (35). Our results demonstrate that PPAR α is also expressed in purified human pancreatic β -cells. Moreover, under conditions of lipotoxicity induced by chronic fatty acid exposure (25,36), different PPAR α agonists (fenofibric acid and ciprofibrate) as well as the PPAR γ 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, PPAR α agonist treatment improves β -cell function also in human islets in vitro under pathological conditions of lipotoxicity. It was recently demonstrated that PPAR α 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 PPAR α 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 PPAR α agonists in the prevention of type 2 diabetes.

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

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