Pioglitazone Reduces Islet Triglyceride Content and Restores Impaired Glucose-Stimulated Insulin Secretion in Heterozygous Peroxisome Proliferator–Activated Receptor-γ–Deficient Mice on a High-Fat Diet

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Heterozygous peroxisome proliferator–activated receptor-γ (PPAR-γ)–deficient (PPARγ−/−) mice were protected from high-fat diet–induced insulin resistance. To determine the impact of systemic reduction of PPAR-γ activity on β-cell function, we investigated insulin secretion in PPARγ−/− mice on a high-fat diet. Glucose-induced insulin secretion in PPARγ+/− mice was impaired in vitro. The tissue triglyceride (TG) content of the white adipose tissue, skeletal muscle, and liver was decreased in PPARγ+/− mice, but it was unexpectedly increased in the islets, and the increased TG content in the islets was associated with decreased glucose oxidation. Administration of a PPAR-γ agonist, pioglitazone, reduced the islet TG content in PPARγ+/− mice on a high-fat diet and ameliorated the impaired insulin secretion in vitro. Our results demonstrate that PPAR-γ protects islets from lipotoxicity by regulating TG partitioning among tissues and that a PPAR-γ agonist can restore impaired insulin secretion under conditions of islet fat accumulation. Diabetes 53:2844–2854, 2004

Type 2 diabetes is considered to be a polygenic disease that is aggravated by environmental factors, such as low physical activity or a hypercaloric lipid-rich diet. Free fatty acids (FFAs) have been shown to influence insulin secretion (1,2) and insulin action (3), and they are now thought to represent an important factor linking excess fat mass to type 2 diabetes (4,5). According to the lipotoxicity hypothesis, chronic exposure to elevated FFA levels impairs β-cell function and is often accompanied by increased islet triglyceride (TG) content (6–10). However, islet TG content per se may not have a direct mechanistic link to the regulation of insulin secretion (11), although it may be a marker.

Peroxisome proliferator–activated receptor-γ (PPAR-γ) is a ligand-activated transcription factor that belongs to the nuclear hormone receptor superfamily and forms a heterodimer with a retinoid X receptor (12–16). PPAR-γ1 is expressed in many tissues and cells, including white and brown adipose tissue, skeletal muscle, intestine, and macrophages, whereas the splice variant PPAR-γ2 is mainly expressed in both white and brown adipose tissue (17–19). PPAR-γ is also expressed in pancreatic β-cells, but its level of expression is much lower than elsewhere (20). Agonist-induced activation of PPAR-γ/retinoid X receptor is known to increase insulin sensitivity (21,22), and synthetic ligands of PPAR-γ, thiazolidinediones, which have the ability to directly bind and activate PPAR-γ (21) and to stimulate adipocyte differentiation (13,23), are used clinically to reduce insulin resistance and improve hyperglycemia in type 2 diabetes.

The DPP (Diabetes Prevention Program) study (24) recently demonstrated that metformin reduced the development of diabetes by 31%. Interestingly, troglitazone, a PPAR-γ agonist, was shown to be even more effective than metformin in preventing diabetes, although the trial was discontinued because of liver toxicity (see National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health website at http://www.niddk.nih.gov/patient/dpp/dpp.htm), and the TRIPOD (Troglitazone in the Prevention of Diabetes) study revealed a 56% reduction in the incidence of type 2 diabetes in women with prior gestational diabetes who received the drug (25). These observations suggest that reducing the demand for insulin secretion as a result of chronic insulin resistance greatly decreases the risk of deterioration to diabetes. Moreover, the results of these studies also raise the possibility that PPAR-γ agonists may prevent the development of diabetes more potently than metformin through some other mechanism (e.g., amelioration of β-cell function) in addition to amelioration of insulin resistance.

We previously reported that heterozygous PPAR-γ–deficient (PPARγ−/−) mice were protected from high-fat...
diet-induced adipocyte hypertrophy, obesity, and insulin resistance (26). Similar results were reported by another group (27,28). We also noted a much lower insulin response during a glucose tolerance test in PPARγ+/− mice on a high-fat diet compared with wild-type mice on a high-fat diet (26), and it appeared to be disproportionate to the degree of amelioration of insulin resistance. Moreover, the Pro12Ala polymorphism in human PPAR-γ2, which moderately reduces the transcriptional activity of PPAR-γ and protects against the development of insulin resistance and type 2 diabetes (29–31), has been shown to be a risk factor for both decreased insulin secretion and disease severity in individuals with type 2 diabetes (32). Furthermore, it was shown that lipid infusion designed to elevate plasma FFA levels resulted in a decrease in insulin secretion during hyperglycemic clamp in carriers of the Ala allele, but it resulted in an increase in control subjects with the wild-type allele (32). This work shows that insulin secretion was impaired in PPARγ−/− mice on the high-fat diet; that the impairment was associated with increased islet TG content; and that pioglitazone, a PPAR-γ agonist, decreased islet TG content and restored the impaired insulin secretion of the PPARγ−/− mice on the high-fat diet. Based on these findings, we propose that a PPAR-γ agonist can restore impaired insulin secretion under conditions of islet fat accumulation.

RESEARCH DESIGN AND METHODS

PPARγ−/− mice (C57BL/6J, CBA, and ICR hybrid background) were generated as described (26). The animals were allowed free access to water, ordinary laboratory diet, or a high-fat diet, and they received standard animal care according to our institutional guidelines. All experiments in this study were performed on male PPARγ+/− and wild-type littermates.

High-fat diet study. The composition of the normal diet (Rodent Diet CE-2; CLEA Japan, Tokyo, Japan) was 50.7% (wt/wt) carbohydrate, 4.0% fat, 25.2% protein, 4.4% dietary fiber, 6.5% crude ash, 3.6% mineral mixture, 1% vitamin mixture, and 4% moisture. The high-fat diet was prepared according to the methods described previously (26,34). The composition of the high-fat diet was 32% safflower oil, 33.1% casein, 17.6% sucrose, 5.6% cellulose, 9.8% mineral mixture, 1.4% vitamin mixture, and 0.5% taurine. The casein, sucrose, vitamin mixture, mineral mixture, and cellulose powder were purchased from Oriental Yeast (Tokyo); safflower oil was from Benibana Food (Tokyo). In some experiments, after 6 weeks on the high-fat diet, pioglitazone (AD-4833-HCl) was administered mixed with the food at a concentration of 0.02% (wt/wt) for the next 4 weeks. Pioglitazone was kindly provided by Takeda Chemical Industries (Osaka, Japan).

Measurement of serum parameters. Glucose, insulin, TG, and FFA levels were determined with a blood glucose meter (Glutest Pro; Sanwa Kagaku Kenkyujo, Nagoya, Japan), by an insulin radioimmunoassay kit (Biotrak; Amersham Pharmacia Biotech, Bucks, U.K.), and with commercial kits (Wako Chemicals, Osaka, Japan), respectively, according to the manufacturer’s instructions.

In vivo glucose homeostasis. For the glucose tolerance test, mice were fasted for 6 h before the study and then loaded with glucose (1.5 mg/g body wt i.p.) (35). Blood samples were collected at different times from the tail vein. For the insulin tolerance test, mice were allowed free access to food and then fasted during the test. They were intraperitoneally challenged with human insulin (0.75 IU/kg body wt, Novolin R; Novo Nordisk, Bagsvaerd, Denmark) (35).

Islet isolation and analysis of insulin secretion and insulin content. The mouse islets were isolated as described previously (36). In brief, after clamping the common bile duct at a point close to the duodenum outlet, 2.5 ml Krebs-Ringer bicarbonate buffer (KRB)-HEPES (129 mmol/l NaCl, 4.8 mmol/l KCl, 1.2 mmol/l MgSO4, 1.2 mmol/l KH2PO4, 2.5 mmol/l CaCl2, 5 mmol/l NaHCO3, and 10 mmol/l HEPES at pH 7.4) containing 0.2% BSA and collagenase (Sigma, St. Louis, MO) was injected into the duct. The swollen pancreas was removed and incubated at 37°C for 3 min. The pancreas was then dispensed by pipetting, and after washing twice with KRB-HEPES, the islets were obtained manually. Insulin secretion was measured with KRB-HEPES containing 0.2% BSA with a basal glucose concentration of 2.8 mmol/l, unless stated otherwise. After preincubation of 10 islets per tube at the basal glucose concentration at 37°C for 20 min, static incubation was performed at 37°C for 1 h. Insulin levels were determined with an radioimmunoassay kit. To examine the effect of co-exposure of islets to FFA and glucose on insulin secretion, we measured insulin secretion by islets from PPARγ−/− mice with KRB-HEPES containing 0.67% (0.1 mmol/l) FFA-free BSA (Sigma) with a glucose concentration of 2.8 or 22.8 mmol/l in the absence or presence of FFA (0.5 mmol/l palmitate; Sigma). Insulin content per isolated islet was measured after extraction with cold acid ethanol.

Determination of DNA content of islets and β-cell mass. We estimated the number of cells per islet based on the islet DNA content. Islet DNA was extracted in Tris-EDTA buffer (10 mmol/l Tris-HCl, 1 mmol/l EDTA at pH 7.5) using an ultrasonicator, and DNA content was measured using PicoGreen reagent (Molecular Probes, Eugene, OR). Immuno histochemical analysis was used to measure β-cell mass as described previously (37,38).

Measurement of tissue TG content. Muscle, liver, and islet homogenates were extracted with 2:1 (vol/vol) chloroform/methanol, and their TG content was determined as described previously (36).

Analysis of changes in gene expression. Comparative analysis of mRNA levels in tissues was performed with fluorescence-based RT-PCR. Total RNA was extracted from tissues with TRIzol (Life Technologies) according to the manufacturer’s instructions and exposed to RNase-free DNase (Nippon Gene, Tokyo). First-strand cDNA was generated by using random 9-mer primers and reverse transcriptase (Takara Shuzo, Kyoto, Japan). The reverse-transcription mixture was amplified with specific primers and an ABI Prism 7900 sequence detector equipped with a thermocycler (Taqman Technology). Primer sequences in Taqman PCR are shown in Table 1.

Glucose oxidation and fatty acid oxidation in isolated islets. Glucose oxidation and fatty acid oxidation were determined as described previously (39–41).

Statistical analysis. Results are expressed as the means ± SE of the results of the number of experiments indicated. Statistical analyses were performed using the Prism software system (GraphPad Software, San Diego, CA). Differences for statistical significance between the two groups were analyzed by Student’s t test for unpaired comparisons. Individual comparisons among more than two experimental groups were assessed with the post hoc Fisher’s projected least significant difference test. Differences were considered significant at P values <0.05.

RESULTS

PPARγ−/− mice showed both increased insulin sensitivity and decreased insulin secretion on the high-fat diet. PPARγ−/− mice and wild-type littermates were fed ordinary laboratory diet (normal diet) until they became 10 weeks of age, and then they were fed normal diet or a high-fat diet. After 20 weeks on the normal diet, PPARγ−/− mice showed body weights similar to wild-type mice (Fig. 1A). By contrast, after 20 weeks on the high-fat diet, wild-type mice had gained significantly more body weight than PPARγ−/− mice (53.6 ± 1.7 vs. 48.6 ± 1.2 g, P = 0.02) (Fig. 1A). On the normal diet, the glucose-lowering effect of insulin was indistinguishable between the two mouse groups (Fig. 1B and C). However, on the high-fat diet, although wild-type mice exhibited insulin resistance compared with other groups, PPARγ−/− mice had insulin sensitivity that was as good as mouse groups on the normal diet (Fig. 1B and C). On the normal diet, wild-type and PPARγ−/− mice showed similar glucose tolerance (Fig. 1D). After 20 weeks on the high-fat diet, wild-type mice developed glucose intolerance during an intraperitoneal glucose tolerance test (Fig. 1D). PPARγ−/− mice also developed glucose intolerance (Fig. 1D), despite having increased insulin sensitivity compared with wild-type mice.
The primers in set 8 were designed to detect both isoforms of PPAR-γ.

**TABLE 1**  
Primer sequences in Taqman PCR

<table>
<thead>
<tr>
<th>Primer set</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  DGAT1-forward primer (F)</td>
<td>TCC GCC TCT GGG CAT TC</td>
</tr>
<tr>
<td>2  DGAT1-reverse primer (R)</td>
<td>GAA TCG GCC CAC AAT CCA</td>
</tr>
<tr>
<td>3  DGAT1-Taqman probe (T)</td>
<td>CCA TGA TGG CTC AGG TGC CAC TGG</td>
</tr>
<tr>
<td>4  FAS-F</td>
<td>CAT TCG TGA TGG AGT CGT GAA</td>
</tr>
<tr>
<td>5  FAS-R</td>
<td>CCA TGT AGC GGA AGG CAT CT</td>
</tr>
<tr>
<td>6  FAS-T</td>
<td>CCC CTC AAG TGC ACA GTG TTT CCC A</td>
</tr>
<tr>
<td>7  CD36-F</td>
<td>GGA TCT GAA ATC GAC CTT AAA GGA</td>
</tr>
<tr>
<td>8  CD36-R</td>
<td>CAG AAA CAA TGG TTG TCT GGA TTC</td>
</tr>
<tr>
<td>9  CD36-T</td>
<td>TGT TCT TCC AGC CAA TGC CTT TGC A</td>
</tr>
<tr>
<td>10 UCP2-F</td>
<td>ACC TCC CTT GCC ACT TCA CTT</td>
</tr>
<tr>
<td>11 UCP2-R</td>
<td>TCT CGT CTT GAC CAC ATC AAC AG</td>
</tr>
<tr>
<td>12 UCP2-T</td>
<td>TCT GCA CCA CCG TCA TGC CCT C</td>
</tr>
<tr>
<td>13 ACS-F</td>
<td>TTG AAA ATA TCT ACT TGG GTA A</td>
</tr>
<tr>
<td>14 ACS-R</td>
<td>CTA TGA GAA AGG CCT GCA AGC TT</td>
</tr>
<tr>
<td>15 ACS-T</td>
<td>CCG TGG CCC AGG TGG TTC TCC A</td>
</tr>
<tr>
<td>16 GPAT-F</td>
<td>GGC TCC TCT CAG TGG TAG TGA ATA</td>
</tr>
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<td>17 GPAT-R</td>
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<tr>
<td>18 GPAT-T</td>
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</tr>
<tr>
<td>19 CPT-I-F</td>
<td>CTT GGG CAT GAT TGC AAA G</td>
</tr>
<tr>
<td>20 CPT-I-R</td>
<td>GAG GAC GCC ACT CAC GAT GT</td>
</tr>
<tr>
<td>21 CPT-I-T</td>
<td>ACC CTA GAC ACC ACT GGC CGC ATG T</td>
</tr>
<tr>
<td>22 PPARγ-F</td>
<td>GGT TTG GGC GGA TGC C</td>
</tr>
<tr>
<td>23 PPARγ-R</td>
<td>GGT GCC GTA TAT CAG TGG AGA TC</td>
</tr>
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<td>24 PPARγ-T</td>
<td>CCG AGA AGG AGA AGC TGT TGG CGG</td>
</tr>
<tr>
<td>25 ACO-F</td>
<td>GGA TTT TCA GAC GCA GTA TAA A</td>
</tr>
<tr>
<td>26 ACO-R</td>
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</tr>
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<td>27 ACO-T</td>
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</tr>
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<td>28 SREBP1a-F</td>
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</tr>
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<td>32 SREBP1c-R</td>
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</tr>
<tr>
<td>33 SREBP1c-T</td>
<td>ACC ACG GAG CCA TGG ATT GCA CATT</td>
</tr>
<tr>
<td>34 Cyclophilin-F</td>
<td>GGT CCT GGC ATC TGG TCC AT</td>
</tr>
<tr>
<td>35 Cyclophilin-R</td>
<td>CAG TCT TGG CAG TGC AGA TAA AA</td>
</tr>
<tr>
<td>36 Cyclophilin-T</td>
<td>CTG GAC CAA ACA CAA AGC GTT CCC A</td>
</tr>
</tbody>
</table>

The primers in set 8 were designed to detect both isoforms of PPAR-γ.

On the high-fat diet (Fig. 1B and C). It should be noted that on the high-fat diet, serum insulin levels before and 30 min after glucose load in PPARγ+/− mice were significantly lower than those in wild-type mice (Fig. 1E), reproducing a similar result obtained from the oral glucose tolerance test (26). Thus, PPARγ+/− mice on the high-fat diet failed to show significantly better glucose tolerance, despite much increased insulin sensitivity, presumably because of a reduced capacity for insulin secretion.

**Isolets isolated from PPARγ+/− mice on the high-fat diet secrete less insulin than the islets of wild-type mice.** We prepared islets of approximately the same size from wild-type and PPARγ+/− mice and measured glucose-stimulated insulin secretion by their islets. In the presence of 2.8 mmol/l glucose or 50 mmol/l KCl, insulin secretion by the islets of both wild-type and PPARγ+/− mice was similar (Fig. 2A). However, in the presence of 22.2 mmol/l glucose, insulin secretion by the islets of PPARγ+/− mice was significantly reduced by 44% compared with wild-type mice. When insulin secretion was evaluated by the insulin secretion rate (secreted insulin/insulin content), similar results were obtained (Fig. 2B). The insulin content per DNA was indistinguishable between the two groups (Fig. 2C). The number of cells per islet, estimated based on the DNA content per islet, and the β-cell mass, estimated by immunostaining the pancreas, were not different in PPARγ+/− and wild-type mice (Fig. 2D and E). Thus, at the high glucose concentration on the high-fat diet, PPARγ+/− mice secreted less insulin than wild-type mice both in vivo and in vitro.

**TG content is decreased in muscle and liver of PPARγ+/− mice on the high-fat diet, but it is increased in islets.** On the high-fat diet, the FFA and TG serum levels in the PPARγ+/− mice were higher than in wild-type mice under fasted conditions (FFA: P < 0.05; TG: P = 0.17) (Fig. 3A and B). Because PPAR-γ regulates tissue partitioning of FFA and hence of TG content among tissues (15), we measured the TG content of the tissues involved in insulin sensitivity and insulin secretion. White adipose tissue (WAT) mass, and hence the TG content of WAT, in the PPARγ+/− mice was reduced by 45% compared with wild-type mice (Fig. 3C), and the TG content of the skeletal muscle and the liver of PPARγ+/− mice was reduced by 73 and 31%, respectively, compared with wild-type mice (Fig. 3D and E). By contrast, islet TG content was ~1.9-fold higher in the PPARγ+/− mice than in the wild-type mice (P < 0.05) (Fig. 3F). These data raise the possibility that the reduction of PPAR-γ activity altered TG partitioning among tissues so that the TG content of muscle and liver was decreased and that of β-cells was increased, resulting in increased insulin sensitivity and decreased insulin secretion, respectively.

**Reduced glucose oxidation in PPARγ+/− mice on the high-fat diet.** According to the glucose-FFA cycle, referred to as the Randle cycle (6), there is a reciprocal relationship between glucose and FFA metabolism in β-cells (7), although the existence of a Randle cycle in islets is controversial. We measured glucose oxidation and fatty acid oxidation in islets. Glucose oxidation by the isolated islets of PPARγ+/− mice was slower in the presence of 2.8 and 22.2 mmol/l glucose compared with wild-type mice (Fig. 3G), and this may lead to reduced ATP
production by β-cells and impaired insulin secretion. By contrast, fatty acid oxidation by islets isolated from PPARγ−/− mice was only minimally increased at 22.2 mmol/l glucose compared with wild-type mice (Fig. 3H). These results suggest that the increased TG content in the islets of PPARγ−/− mice is not associated with a significant increase in fatty acid oxidation in islets.

**Expression of genes in islets involved in lipid metabolism.** To determine the mechanism responsible for the increased TG content of the islets of PPARγ−/− mice, we investigated the expression of genes involved in fatty acid metabolism. We first investigated the level of PPAR-γ mRNA expression in these tissues by real-time RT-PCR analysis, and the results showed the following order of expression: WAT > muscle > liver > islets. The expression of PPAR-γ mRNA in islets was far lower than in other tissues, and the levels of expression in WAT, muscle, liver, and islets in PPARγ−/− mice were 50–79% lower than in wild-type mice (Table 2). FFA is transported into tissues via fatty acid transporters, such as CD36, which is regulated by both PPAR-γ and -α. We previously reported that PPARγ−/− mice showed markedly reduced CD36 expression in WAT and skeletal muscle, in both of which PPAR-γ is relatively abundantly expressed (42). In the liver, where PPAR-γ is less abundantly expressed and PPAR-α is abundantly expressed, the increased PPAR-α activity, presumably caused by increased leptin and adiponectin expression in PPARγ−/− mice, led to the increased CD36 expression. We have also reported that the expression levels of molecules in the β-oxidation pathway, such as acyl-CoA oxidase, are upregulated in PPARγ−/− mice because of increased PPAR-α activity, and that molecules in the lipogenic pathway, such as sterol regulatory element–binding protein-1, are downregulated because of increased leptin action in WAT, skeletal muscle, and liver (42). In the present study we measured the expression levels of genes involved in lipid metabolism in islets. In contrast to WAT, skeletal muscle, and liver, the islet expression levels of sterol regulatory element–binding protein-1α and -1c, which are key transcriptional factors in the regulation of expression of lipogenic enzymes, and the expression levels of molecules in lipogenic pathways, such as acetyl-CoA synthase, fatty acid synthase, and acyl-CoA: diacylglycerol transferase, were similar in PPARγ−/− and
wild-type mice (Table 3). Moreover, in contrast to WAT, skeletal muscle, and liver, CD36 and acyl-CoA oxidase expression in islets was comparable in both groups. We speculate that CD36 expression was not reduced in the islets in PPARγ–/– mice, probably because the contribution of PPAR-γ to FFA uptake appears to be small due to its extremely low level of expression. Because the serum FFA levels of PPARγ–/– mice were significantly elevated, presumably due to reduced uptake by WAT and skeletal muscles, the net influx of FFA into the islets of the PPARγ–/– mice may have been increased, leading to a marked increase in the TG content of their islets.

**PPAR-γ agonist decreases serum FFA levels and islet TG content of PPARγ–/– mice and ameliorates their impaired insulin secretion and glucose metabolism in islets.** We next investigated whether increasing PPAR-γ activity would restore the impaired insulin secretion of PPARγ–/– mice on the high-fat diet by administering the PPAR-γ agonist pioglitazone to wild-type and PPARγ–/– mice on the high-fat diet. Pioglitazone caused weight gain in both groups (Fig. 4A), but it improved glucose tolerance during an intraperitoneal glucose tolerance test in both genotypes (Fig. 4B). It should be noted that although glucose levels 30 min after the glucose load were indistinguishable in wild-type and PPARγ–/– mice on the high-fat diet, those were significantly lower in PPARγ–/– mice with pioglitazone than in wild-type mice with pioglitazone. Serum insulin levels before and after the glucose load were decreased in wild-type mice by pioglitazone (Fig. 4C), suggesting that increased insulin sensitivity rather than increased insulin secretion caused improvement of glucose tolerance by pioglitazone. By contrast, serum insulin levels before and after the glucose load were unaffected by pioglitazone in PPARγ–/– mice (Fig. 4C), raising a possibility that decreased insulin secretion in PPARγ–/– mice was partially restored by pioglitazone. Pioglitazone did not alter serum FFA and TG levels of wild-type mice, whereas it significantly decreased the serum FFA and TG levels of PPARγ–/– mice (Fig. 4D and E).

![FIG. 2.](image-url)
We next studied islet function. The TG content of the islets of the PPARγ−/− mice was significantly decreased by pioglitazone (Fig. 5A). Insulin secretion at 22.2 mmol/l glucose was significantly improved by pioglitazone in PPARγ−/− mice (Fig. 5B), and glucose oxidation in the islets was improved by pioglitazone in PPARγ−/− mice (Fig. 5C). These results indicate that in the PPARγ−/− mice, increased PPAR-γ activity by pioglitazone ameliorated their impaired insulin secretion and glucose metabolism in vitro.

It should be noted that pioglitazone did not change insulin secretion in high-fat diet–fed PPARγ−/− mice in vivo (Fig. 4C), but that insulin secretion was enhanced in vivo and in vitro.

We next studied islet function. The TG content of the islets of the PPARγ−/− mice was significantly decreased by pioglitazone (Fig. 5A). Insulin secretion at 22.2 mmol/l glucose was significantly improved by pioglitazone in PPARγ−/− mice (Fig. 5B), and glucose oxidation in the islets was improved by pioglitazone in PPARγ−/− mice (Fig. 5C). These results indicate that in the PPARγ−/− mice, increased PPAR-γ activity by pioglitazone ameliorated their impaired insulin secretion and glucose metabolism in vitro.

It should be noted that pioglitazone did not change insulin secretion in high-fat diet–fed PPARγ−/− mice in vivo (Fig. 4C), but that insulin secretion was enhanced in vivo and in vitro.

TABLE 2
Relative mRNA expression levels of PPAR-γ in WAT, muscle, liver, and islets of wild-type mice and PPARγ−/− mice on the high-fat diet. PPAR-γ mRNA expression in islets was by far lower than in other tissues.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>WAT</th>
<th>Muscle</th>
<th>Liver</th>
<th>Islets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>100</td>
<td>23.3</td>
<td>1.90</td>
<td>0.18</td>
</tr>
<tr>
<td>PPARγ−/−</td>
<td>20.9 (20.9)</td>
<td>9.0 (38.6)</td>
<td>0.71 (37.4)</td>
<td>0.09 (50.0)</td>
</tr>
</tbody>
</table>

Values represent PPAR-γ mRNA levels normalized by cyclophilin mRNA and are expressed as percentages of the values in wild-type WAT. The numbers in parentheses are percentages of the values in wild-type mice.
vitro (Fig. 5B). One possibility is that the lipid-lowering effect of pioglitazone resulted in a decreased ambient FFA concentration for potentiation of glucose-stimulated insulin secretion (Fig. 4D) (43). Thus, we examined the effect of co-exposure to FFA and glucose on insulin secretion in islets from PPARγ/H9253/H11001/H11002 mice by incubating islets in vitro with glucose (2.8 or 22.2 mmol/l) plus FFA (0.5 mmol/l palmitate) (Fig. 5D). Exposure to circulating palmitate potentiated high-glucose–stimulated insulin secretion, but not low-glucose–stimulated insulin secretion, in islets from PPARγ/H9253/H11001/H11002 mice. Thus, glucose-stimulated insulin secretion in vivo from pioglitazone-treated PPARγ/H9253/H11001/H11002 mice was likely to be underestimated as compared with that from untreated PPARγ+/− mice because pioglitazone significantly decreased the ambient FFA concentration (Fig. 4D). Of note, this discrepancy between in vitro and in vivo was not the case in wild-type mice, presumably at least in part because of unaltered ambient FFA (Fig. 4D).

The values of insulin secretion in PPARγ+/− islets after high-fat diet (Fig. 5B) were markedly different from those presented in Fig. 2A, especially in terms of the fold increase. Similarly, the levels of glucose oxidation between wild-type and PPARγ+/− islets were no longer significant in Fig. 5C, whereas the values were significantly different in Fig. 3G. We interpreted the discrepancy between the results (Figs. 2A vs. 5B, 3G vs. 5C) because of...
the duration of high-fat diet. Thus, the results in Fig. 2 or 3 were obtained from mouse groups 20 weeks after starting the high-fat diet, whereas those in Fig. 5 were obtained from mouse groups 6 weeks after starting the high-fat diet, followed by 4 weeks without pioglitazone treatment, in other words only 10 weeks after starting the high-fat diet.

**DISCUSSION**

These results show that although moderate reduction of PPAR-γ activity increased insulin sensitivity in PPARγ+/− mice on the high-fat diet, insulin secretion was impaired, and the impairment was apparently associated with increased islet TG content and decreased glucose oxidation. We previously showed that a moderate reduction of PPAR-γ activity in PPARγ−/− mice decreased the TG content of WAT, muscle, and liver, thereby ameliorating high-fat diet–induced obesity and insulin resistance (42). The islet TG content of the PPARγ+/− mice on the high-fat diet, however, was increased. We hypothesize that islet TG content is determined by partitioning among various tissues, independent of peripheral insulin sensitivity. Figure 6A shows our model for the mechanisms of the increased insulin sensitivity and decreased insulin secretion of PPARγ+/− mice on the high-fat diet. FFA is transported into tissues via fatty acid transporters, such as CD36, which is regulated by both PPAR-γ and -α. PPARγ+/− mice showed markedly reduced CD36 expression in WAT and skeletal muscle, in both of which PPAR-γ is relatively abundantly expressed, and as a result, these tissues showed reduced FFA uptake and markedly reduced TG content in PPARγ+/− mice. In the liver, where PPAR-γ is less abundantly expressed and PPAR-α is abundantly expressed, increased PPAR-α activity, presumably caused by increased adiponectin expression and secretion in the WAT of PPARγ+/− mice, led to the increase in CD36 expression (42). Interestingly, despite increased FFA uptake by the liver, tissue TG content was moderately reduced because of increased β-oxidation of lipids as a result of increased PPAR-α activity. CD36 expression was not reduced in the islets of PPARγ+/− mice probably because the contribution of PPAR-γ to FFA uptake is likely to be small, given the extremely low expression of PPAR-γ. Because the serum FFA levels were significantly elevated in PPARγ+/− mice, presumably due to reduced uptake by WAT and skeletal muscle, the net influx of FFA into the islets may have been increased. Moreover, the enzymes involved in β-oxidation, such as acyl-CoA oxidase, were not upregulated in the islets of PPARγ+/− mice (Table 3). Thus, the combination of increased FFA uptake and no compensatory increase in β-oxidation pathway may have caused a marked increase in the TG content in the islets of PPARγ+/− mice.

Thus, our model proposes that the PPAR-γ activity in β-cells does not directly regulate β-cell function, but that PPAR-γ activity in other tissues determines the islet TG content indirectly. Consistent with our model, insulin secretion in β-cell–specific PPAR-γ knockout mice on a high-fat diet has been reported to be normal (44).

Pioglitazone improved insulin resistance and glucose tolerance in both genotypes on the high-fat diet in our study, and it restored the impaired insulin secretion in PPARγ+/− mice. It is conceivable that the reduction in demand for insulin secretion due to chronic insulin resistance by pioglitazone greatly decreased the excess stimulation of β-cells to release insulin. However, this appears to be contradicted by the fact that the same dose of

FIG. 5. Effect of PPAR-γ agonist on islet function of PPARγ+/− mice on the high-fat diet. A: Effect of pioglitazone on islet TG content in wild-type mice and PPARγ+/− mice after 4 weeks with or without pioglitazone. Values are expressed as the means ± SE (n = 4–6). B and C: Effect of pioglitazone on the rate of insulin secretion per islet during static incubation (B) and glucose oxidation (C) by isolated islets. Islets were isolated from wild-type mice and PPARγ+/− mice after 4 weeks with or without pioglitazone. Values are expressed as the means ± SE (n = 4–5). D: Effect of co-exposure of PPARγ−/− islets to FFA and glucose during static incubation by isolated islets. Islets were isolated from wild-type mice and PPARγ+/− mice after 20 weeks on the high-fat diet. The results are shown as nanograms insulin per islet per hour (n = 4). *P < 0.05; **P < 0.01. □, wild-type mice, high-fat diet; □, wild-type mice, high-fat diet plus pioglitazone; □, PPARγ−/−, high-fat diet; □, PPARγ−/−, high-fat diet plus pioglitazone.
FIG. 6. Possible mechanisms of the increased insulin sensitivity and decreased insulin secretion in \( PPAR\gamma^{-/-} \) mice on the high-fat diet and restored insulin secretion after pioglitazone treatment. 

**A:** In wild-type mice, most FFAs derived from the high-fat diet are transported into WAT, where PPAR-\( \gamma \) is abundantly expressed, and some of the FFAs are transported into muscle and liver, where PPAR-\( \gamma \) is moderately expressed. Consequently, FFA transport into islets, where PPAR-\( \gamma \) is hardly expressed, may be suppressed. In \( PPAR\gamma^{-/-} \) mice, FFA transport into WAT and muscle is limited because of the reduced expression of CD36 (42). Although FFA transport into liver is increased, TG content is decreased because of increased FFA oxidation in liver. Consequently, the decreased TG content in these tissues may ameliorate insulin resistance. On the other hand, islet TG content is increased as a result of elevated level of serum FFAs, which are not transported into WAT, muscle, and liver. Thus, decreased insulin secretion can be caused by lipotoxicity. 

**B:** Serum FFA levels were significantly reduced in \( PPAR\gamma^{-/-} \) mice by pioglitazone, presumably because of increased uptake by WAT. This alteration in TG partitioning resulted in the decreased net influx of FFA into the islets, thereby protecting against overaccumulation of lipids in the islets. Consequently, the impaired insulin secretion of \( PPAR\gamma^{-/-} \) mice on the high-fat diet was restored by pioglitazone. ACO, acyl-CoA oxidase.
pioglitazone ameliorated insulin resistance on the high-fat diet in both genotypes (Fig. 4B), but it improved insulin secretion only in the PPARγ+/− mice, not in the wild-type mice (Fig. 5B). The fact that pioglitazone decreased islet TG content in PPARγ+/− mice but not in wild-type mice (Fig. 5A) suggests a link between decreased islet TG content and restored insulin secretion in PPARγ+/− mice (Fig. 6B), but we have no evidence on the cause-and-effect relationship between the islet TG content and insulin secretion. Moreover, it was recently reported that there is no clear mechanistic link between islet TG content and insulin secretion (11). Accordingly, we cannot conclude that islet TG content per se has anything to do with the regulation of insulin secretion directly, although it may be a marker.

What, then, is the mechanism of the pioglitazone-induced reduction in islet TG content in PPARγ+/− mice? Serum FFA levels were significantly reduced in PPARγ+/− mice by pioglitazone (Fig. 4D), presumably because of increased uptake by WAT, as evidenced by increased body weight (Fig. 4A). The alteration in TG partitioning resulted in the decreased net influx of FFAs into the islets, thereby protecting against overaccumulation of lipids in the islets. Thus, pioglitazone regulates TG partitioning among WAT, skeletal muscle, liver, and the islets, independent of peripheral insulin resistance. Shimabukuro et al. (45), however, observed that troglitazone decreased esterification and increased oxidation of fatty acids in the islets of ZDF rats in vitro, thereby decreasing the excessive TG content of the islets and restoring insulin secretion. Moreover, Lupi et al. (46) recently showed that rosiglitazone prevented the impairment of human islet function induced by fatty acids in vitro. Interestingly, it was operative mainly under conditions of islet fat accretion or increased fatty acid availability. Given the extremely low expression of PPAR-γ in mouse islets (Table 2) and no compensatory increase in β-oxidation pathway (Table 3), however, this direct mechanism may not fully explain the pioglitazone-induced reduction in islet TG content. Although the mechanism linking decreased islet TG content and improved insulin secretion by pioglitazone has not been fully elucidated, it is very interesting that this action is operative under conditions of fat overaccumulation in islets.

In summary, our results clearly demonstrate that PPAR-γ protects islets from lipotoxicity by regulating TG partitioning among tissues, and that pioglitazone can restore insulin secretion impaired by lipotoxicity. These findings, together with the results of the study showing that troglitazone prevents diabetes (25), suggest that PPAR-γ agonists protect β-cells from insulin resistance and lipotoxicity by regulating TG partitioning among various tissues, independent of peripheral insulin resistance.

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