

## Targeted Elimination of Peroxisome Proliferator-Activated Receptor $\gamma$ in $\beta$ Cells Leads to Abnormalities in Islet Mass without Compromising Glucose Homeostasis

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**The nuclear hormone receptor peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) is an important regulator of lipid and glucose homeostasis and cellular differentiation. Studies of many cell types *in vitro* and *in vivo* have demonstrated that activation of PPAR $\gamma$  can reduce cellular proliferation. We show here that activation of PPAR $\gamma$  is sufficient to reduce the proliferation of cultured insulinoma cell lines. We created a model with mice in which the expression of the *PPARG* gene in  $\beta$  cells was eliminated ( $\beta\gamma$ KO mice), and these mice were found to have significant islet hyperplasia on a chow diet. Interestingly, the normal expansion of  $\beta$ -cell mass that occurs in control mice in response to high-fat feeding is markedly blunted in these animals. Despite this alteration in  $\beta$ -cell mass, no effect on glucose homeostasis in  $\beta\gamma$ KO mice was noted. Additionally, while thiazolidinediones enhanced insulin secretion from cultured wild-type islets, administration of rosiglitazone to insulin-resistant control and  $\beta\gamma$ KO mice revealed that PPAR $\gamma$  in  $\beta$  cells is not required for the antidiabetic actions of these compounds. These data demonstrate a critical physiological role for PPAR $\gamma$  function in  $\beta$ -cell proliferation and also indicate that the mechanisms controlling  $\beta$ -cell hyperplasia in obesity are different from those that regulate baseline cell mass in the islet.**

Peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$  [NR1C3], encoded by *PPARG*) is a member of the nuclear hormone receptor superfamily of ligand-gated transcription factors. PPAR $\gamma$  has been shown to play a role in diverse biological processes, including adipogenesis and glucose and lipid homeostasis (33). Although the endogenous ligand of PPAR $\gamma$  is not known, there are several synthetic compounds that bind PPAR $\gamma$  with high affinity and activate the receptor. These include the thiazolidinedione (TZD) class of drugs, which are insulin sensitizers currently in use for the treatment of type 2 diabetes.

In addition to insulin sensitization, other actions of PPAR $\gamma$  have recently come to light, including the control of cellular proliferation. Activation of PPAR $\gamma$  has been shown to reduce growth in a variety of cell lines cultured from cancers of adipose tissue (40), colon (35), breast (29), prostate (30), liver (34), lung (41), and pancreatic acinar tissue (13). These findings have been extended to some human tumors, such as liposarcoma (11) and prostate cancer (30), and clinical trials with

other forms of cancer are in progress. Interestingly, loss-of-function mutations in *PPARG* have been demonstrated for some human colon cancers (36) and loss of heterozygosity at 3p25, a broad region that includes *PPARG*, has been seen in many human prostate (30) and pancreatic endocrine (9, 10, 31, 38, 46) tumors.

The latter observation led us to wonder whether the activation of PPAR $\gamma$  in pancreatic  $\beta$  cells would also regulate cellular proliferation. PPAR $\gamma$  is expressed in the  $\beta$  cells of both rodents and humans (12, 47), and treatment of diabetic or prediabetic humans and rodents with PPAR $\gamma$  agonists leads to improvements in islet architecture, insulin content, and glucose-stimulated insulin secretion (GSIS) (6, 8, 17). Although these effects could be explained as the beneficial sequelae of reducing peripheral insulin resistance, troglitazone added directly to the culture medium of islets isolated from Zucker fatty rats also improves GSIS (37). Additionally, activation of PPAR $\gamma$  directly induces the expression of the glucose transporter Glut2 and glucokinase, critical participants in GSIS, in cultured primary rat islets and insulinoma cell lines (20, 21).

These studies suggested to us that PPAR $\gamma$  may exert a significant effect on  $\beta$ -cell function and that at least part of the therapeutic effect of TZD administration may be mediated through the pancreatic islet. We therefore sought to determine whether PPAR $\gamma$  could regulate the growth and function of  $\beta$  cells by using both cultured insulinoma cell lines and targeted gene elimination in mice.

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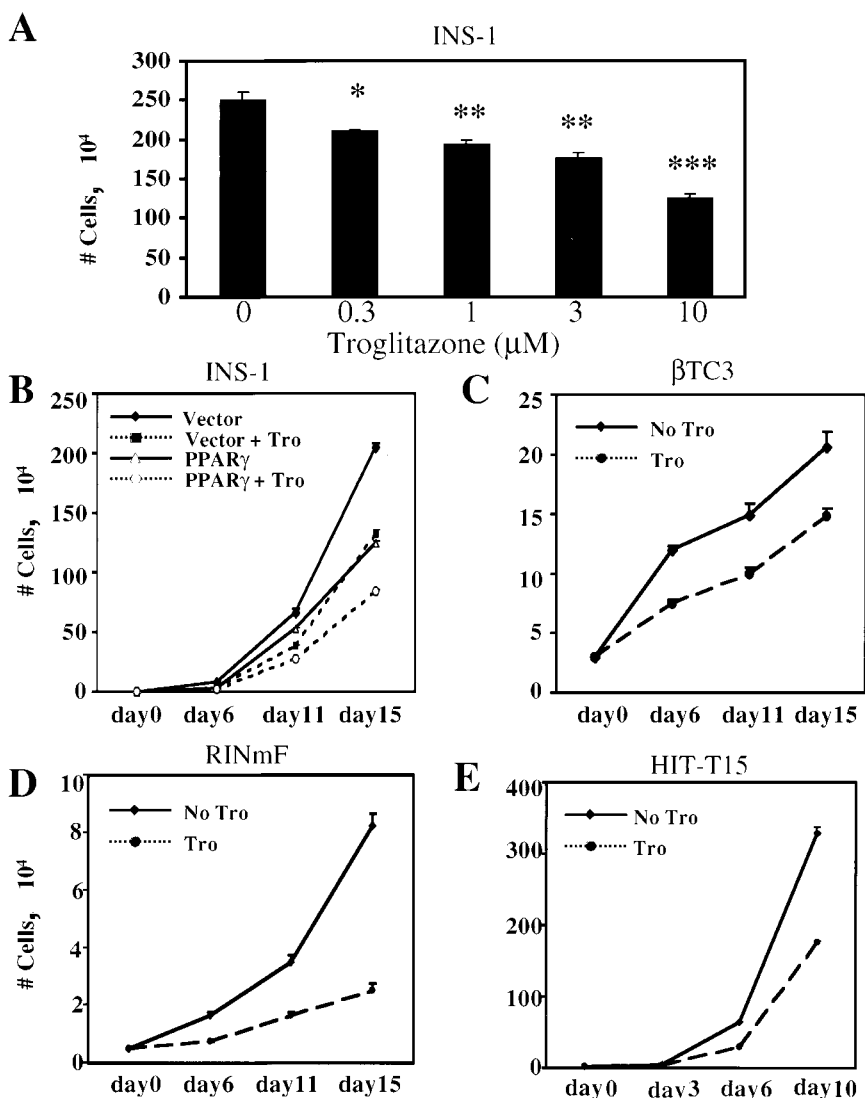


FIG. 1. Effect of TZDs on cultured insulinoma cell growth. (A) INS-1 cells were treated with various doses of troglitazone for 15 days before being counted. Results are given as mean numbers of cells ( $10^4$ )  $\pm$  standard errors of the means from at least three independent experiments. \*,  $P < 0.05$ ; \*\*,  $P < 0.005$ ; \*\*\*,  $P < 0.001$ . (B) INS-1 cells were infected with a retrovirus expressing PPAR $\gamma$ 1 or vector only and then treated with vehicle (solid lines) or 10  $\mu$ M troglitazone (Tro) (dashed lines) before being counted as described for panel A. (C to E)  $\beta$ TC3, RINmF, and HIT-T15 cells were cultured in the presence of 10  $\mu$ M troglitazone or vehicle for the indicated numbers of days before being counted as described for panel A.

**MATERIALS AND METHODS**

**Animal protocols.** Study populations of mice were generated by crossing animals with exon 2 of *PPARG* flanked by loxP sites (PPAR $\gamma^{fl/fl}$ ) to mice expressing Cre recombinase driven by the rat insulin promoter (PPAR $\gamma^{cre}$ ). F<sub>1</sub> PPAR $\gamma^{fl/+}$  mice were then crossed with F<sub>1</sub> PPAR $\gamma^{fl/+ cre}$  mice, thus generating the four study groups (PPAR $\gamma^{fl/fl}$ , PPAR $\gamma^{+/+}$ , PPAR $\gamma^{+/+ cre}$ , and PPAR $\gamma^{fl/fl cre}$  [heterofores and herein called  $\beta\gamma$ KO]). Because mice were maintained on a mixed FVB-129-C57 background, littermates served as controls for all experiments. For studies involving a high-fat diet, mice were fed Harlan Teklad TD93075 special diet (55% fat by caloric content). Mice were genotyped by PCR using the following primers (see Fig. 2): F, CTCCAATGTTCTCAAACCTTAC; R1, GATGAGTCATGTAAGTTGACC; and R2, GTATTCTATGGCTCCAGTGC. All animal work was performed with the approval of the Animal Use Committees of Harvard and Brandeis Universities.

**Blood glucose, plasma insulin, glucose tolerance tests, and in vivo insulin secretion.** Blood glucose values were determined from whole venous blood by using an automated glucose monitor (Glucometer Elite; Bayer). Insulin levels in serum were measured by enzyme-linked immunosorbent assay using mouse in-

sulin as a standard (Crystal Chem, Chicago, Ill.). Glucose tolerance tests and acute insulin secretion tests were performed on animals that had been fasted overnight for 16 h. The peritoneal cavities of the animals were injected with 2 g of glucose/kg of body weight. Glucose levels were measured from blood collected from the animals' tails immediately before and 15, 30, 60, and 120 min after the injections (24).

**Islet morphology and immunohistochemistry.** Tissues were fixed in Bouin's solution and 10% buffered formalin and embedded in paraffin. Sections of pancreas were stained for non- $\beta$ -cell hormones with a cocktail of antibodies to glucagon, somatostatin, and pancreatic polypeptide (24, 27). Immunofluorescent staining for insulin was achieved with anti-insulin antibodies and detected by fluorescein antibodies (Jackson ImmunoResearch).  $\beta$ -Cell growth was evaluated by bromodeoxyuridine (BrdU) labeling. Mice were injected with BrdU (Sigma) ~6 h before sacrifice, followed by dissection of the pancreas for staining and sectioning as described above. BrdU-positive cells were identified with an anti-BrdU antibody (cell proliferation kit; Amersham, Piscataway, N.J.).

**$\beta$ -cell mass determination.**  $\beta$ -Cell mass was evaluated by point counting morphometry on immunoperoxidase-stained sections of pancreas (27, 28). Multiple

sections (separated by 80  $\mu\text{m}$  each) were obtained from each pancreas and analyzed systematically by using a grid system covering at least 175 fields per mouse. Separate images were acquired with a BX60 microscope (Olympus, New Hyde Park, N.Y.) as described previously (28). Relative volumes were calculated for  $\beta$  cells, non- $\beta$  cells, and exocrine tissue. The extent of contaminating tissue (pancreatic ducts, lymph nodes, adipose, and intestine) was recorded to correct for the pancreatic weight.  $\beta$ -Cell mass was calculated by the following formula: islet  $\beta$ -cell mass = relative  $\beta$ -cell volume  $\times$  corrected pancreatic weight.

**Fluorescence sorting of islet cells.** We used the observation that glucose stimulation promotes greater intracellular calcium concentration in  $\beta$  cells than in non- $\beta$  cells to sort islet cells (4, 18). Briefly, islets were dispersed after isolation to obtain single cells as described previously (19). Dispersed cells were suspended in Krebs-Ringer buffer (KRB) containing 0.1 mM glucose, 2  $\mu\text{M}$  Fluo-4, and 0.02% Pluronic F-127 (Molecular Probes, Eugene, Ore.) and incubated for 30 min at 37°C. Cells were then washed with 0.1 mM glucose KRB for 30 min, followed by stimulation with 20 mM glucose for 10 min. Samples were washed and kept at 37°C until analysis. Flow cytometry was used for sorting (excitation, 488 nm; emission, 502 to 542 nm), and islet cells were separated based on low (non- $\beta$  cells) versus high ( $\beta$  cells) fluorescence. We extracted RNA from cell pellets and analyzed gene expression by reverse transcription-PCR (RT-PCR). We used nested PCR for amplifying PPAR $\gamma$  cDNA. Primer details are available on request.

**Insulin release and content of islets.** Islets were isolated by using the intraductal collagenase method as described previously (27). The insulin released in vitro from isolated islets was measured by static incubation of islets cultured overnight (27). Insulin content in whole pancreas in acid-ethanol extracts was measured by enzyme-linked immunosorbent assay (Crystal Chem).

**Cell culture and proliferation experiments.** INS-1 and HIT-T15 cells were grown in RPMI 1640 with 10% fetal bovine serum, 0.5 M HEPES, 102 mM L-glutamine, 50 mM sodium pyruvate, and 2.5 mM  $\beta$ -mercaptoethanol. RINmF and  $\beta$ TC3 cells were grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum. Cells were plated on day 0 and counted with a hemocytometer on the days indicated in Fig 1. Troglitazone (10  $\mu\text{M}$ ) or vehicle (0.1% dimethyl sulfoxide) was added as appropriate. Retroviral supernatants expressing PPAR $\gamma$ 1 or vector (pMSCVpuro) only were generated, and INS-1 cells were infected and selected in puromycin as described previously (32).

## RESULTS

**PPAR $\gamma$  regulates cellular proliferation in cultured insulinoma cells.** We first assessed the effect of PPAR $\gamma$  activation on the proliferation of insulinoma cells in vitro. Rat INS-1 cells were treated with various doses of troglitazone or vehicle for 15 days, and cell numbers were counted; a dose-dependent reduction in cellular proliferation was noted (Fig. 1A). Inhibition of cellular proliferation could be seen with as little as 0.03  $\mu\text{M}$  troglitazone, significantly below the  $K_d$  of the receptor for this ligand, making a nonspecific effect of troglitazone very unlikely. To modulate PPAR $\gamma$  activity in an independent way, we also expressed the receptor by retrovirally mediated gene transfer into INS-1 cells. This step had an effect identical to that of ligand administration, and the combination of PPAR $\gamma$  and ligand caused an additive repression of cell growth (Fig. 1B). This phenomenon is not restricted to INS-1 cells, as other  $\beta$ -cell-derived lines ( $\beta$ TC3, HIT-T15, and RINmF) responded similarly to the addition of 10  $\mu\text{M}$  troglitazone (Fig. 1C to E).

**PPAR $\gamma$  regulates cellular proliferation in islets in vivo.** The data above show that PPAR $\gamma$  exerts strong antiproliferative effects on  $\beta$  cells when it is expressed via viral vectors or activated by ligand. If this effect of PPAR $\gamma$  is biologically important, then the absence of the receptor in  $\beta$  cells may lead to islet hyperplasia. Unfortunately, this hypothesis could not be tested with a global PPAR $\gamma$  knockout model, as these mice die early in embryogenesis (5, 22). To test our hypothesis, we crossed mice bearing a version of *PPARG* with loxP sites flanking exon 2 (PPAR $\gamma^{\text{fl/fl}}$ ) (Fig. 2A) with mice expressing Cre

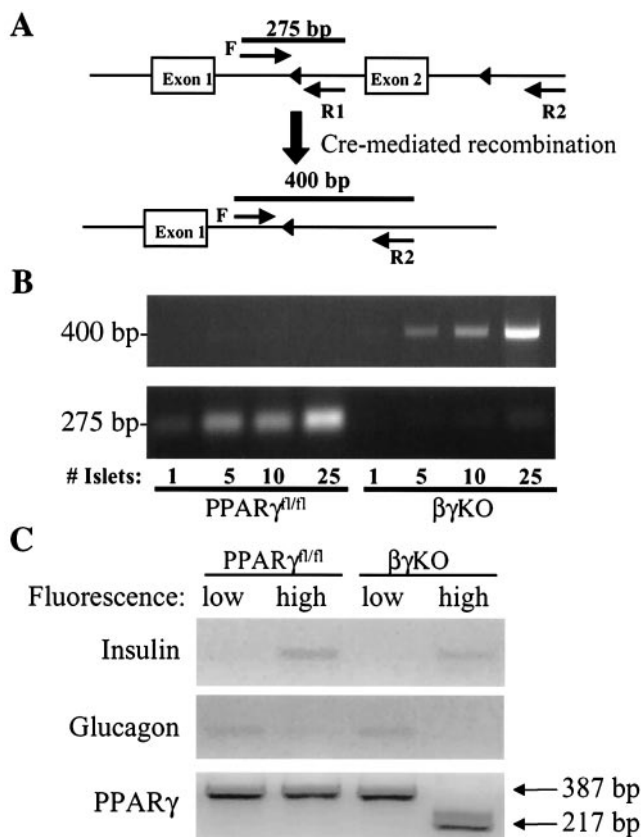


FIG. 2. Deletion of *PPARG* exon 2 in  $\beta\gamma\text{KO}$  mice. (A) Scheme showing exon 2 of *PPARG* targeted with loxP sites (triangles) before and after Cre-mediated recombination. Heavy lines indicate the expected PCR products from primers F, R1, and R2. The 400-bp band is a specific recombination product seen only when exon 2 is appropriately excised, while the 275-bp band represents the nonrecombined floxed allele. (B) Results of PCR from whole islets isolated from PPAR $\gamma^{\text{fl/fl}}$  and  $\beta\gamma\text{KO}$  mice. (C) Dispersed islet cells were loaded with a calcium-sensitive fluorophore and treated with glucose, followed by flow sorting. RT-PCR for insulin and glucagon shows that cells with high fluorescence are primarily  $\beta$  cells but that those with low fluorescence are primarily non- $\beta$  cells. RT-PCR of RNA from these cells yields a band of 387 bp for the wild-type transcript and 217 bp for the recombined transcript missing exon 2.

recombinase from a  $\beta$ -cell-specific promoter (rat insulin promoter [RIP]-Cre). Both of these lines have been described previously (2, 15). F<sub>1</sub> PPAR $\gamma^{\text{fl/+}}$  mice with and without Cre were mated to one another to generate PPAR $\gamma^{\text{fl/fl}}$ , PPAR $\gamma^{+/+}$ , PPAR $\gamma^{\text{cre}}$ , and  $\beta\gamma\text{KO}$  animals.  $\beta\gamma\text{KO}$  mice were born in the expected Mendelian ratio and were not found to have a survival advantage or disadvantage during more than 2 years of study. Figure 2B shows the results of whole-islet PCR demonstrating that the recombined allele (represented by the 400-bp band) appears only in  $\beta\gamma\text{KO}$  mice, as expected. The 275-bp band represents the nonrecombined floxed allele, present as expected in the PPAR $\gamma^{\text{fl/fl}}$  mice but virtually absent from  $\beta\gamma\text{KO}$  mice. As the number of islets increases, a small amount of nonrecombined product in the  $\beta\gamma\text{KO}$  mice becomes apparent, most likely reflecting the presence of non- $\beta$  cells, which do not express the RIP-Cre transgene. In order to ascertain whether PPAR $\gamma$  mRNA reflected the recombination event in

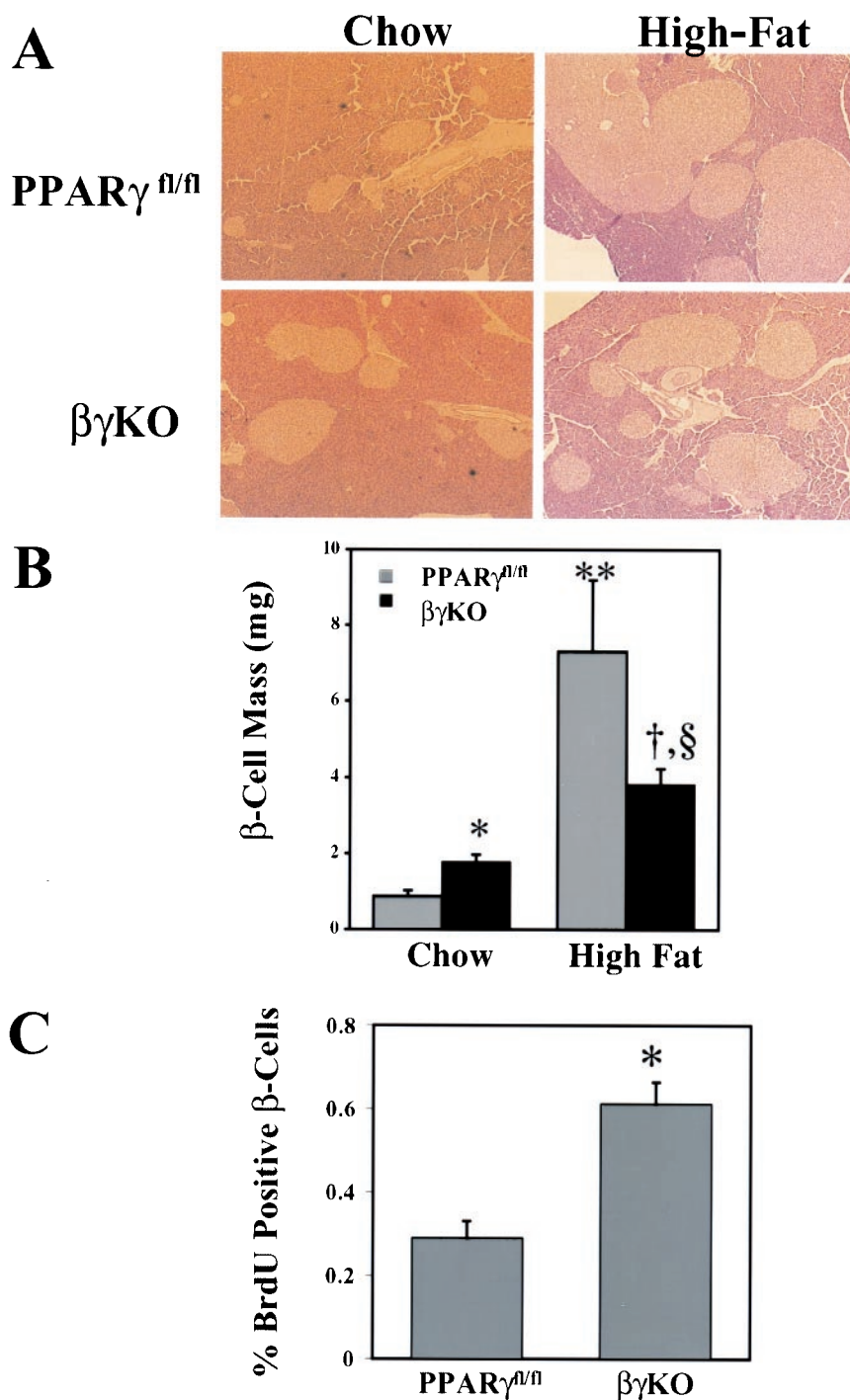


FIG. 3. Islet morphology and histomorphometry in control and  $\beta\gamma$ KO mice. (A) Hematoxylin and eosin staining of pancreata from PPAR $\gamma^{fl/fl}$  and  $\beta\gamma$ KO mice maintained on either a chow or high-fat diet. (B) Quantification of  $\beta$ -cell mass from PPAR $\gamma^{fl/fl}$  and  $\beta\gamma$ KO mice maintained on either a chow or high-fat diet. Four mice were tested for each determination. \*,  $P < 0.05$  for PPAR $\gamma^{fl/fl}$  versus  $\beta\gamma$ KO mice on the chow diet; \*\*,  $P < 0.001$  for PPAR $\gamma^{fl/fl}$  mice on the chow versus the high-fat diet; §,  $P < 0.001$  for  $\beta\gamma$ KO mice on the chow versus the high-fat diet; †,  $P < 0.001$  for PPAR $\gamma^{fl/fl}$  versus  $\beta\gamma$ KO mice on the high-fat diet. (C) BrdU labeling of islets from chow-fed control and  $\beta\gamma$ KO mice (\*,  $P < 0.01$ ;  $n = 4$ ).

$\beta$  cells, we took advantage of the observation that intracellular calcium levels are higher in  $\beta$  cells than in non- $\beta$  cells after a glucose challenge (4, 18). This difference allows one to separate  $\beta$  cells from non- $\beta$  cells using flow sorting on the basis of fluorescence in the presence of a calcium-activated fluoro-

phore and glucose. Figure 2C shows that dispersed islet cells characterized by high and low fluorescence were characterized by the presence of insulin and glucagon mRNA, respectively. Using RT-PCR primers for PPAR $\gamma$  complementary to exons 1 and 3, we show that there is virtually no recombination of

TABLE 1. Glucose and insulin levels in the sera of  $\beta\gamma$ KO and control mice<sup>a</sup>

Phenotype	Glucose (mg/dl)				Insulin (ng/ml)			
	Fasting <sup>b</sup>		Fed		Fasting		Fed	
	Chow diet (n)	High-fat diet (n)	Chow diet	High-fat diet	Chow diet	High-fat diet	Chow diet	High-fat diet
PPAR $\gamma^{+/+}$	76 $\pm$ 5.1 (13)	120 $\pm$ 11.8 (6)	138 $\pm$ 7.2	142 $\pm$ 2.2	448 $\pm$ 180	6,191 $\pm$ 1,356	2,518 $\pm$ 896	ND
PPAR $\gamma^{fl/fl}$	87 $\pm$ 6.9 (6)	111 $\pm$ 7.5 (14)	147 $\pm$ 7.6	138 $\pm$ 9.9	256 $\pm$ 50	6,202 $\pm$ 878	1,682 $\pm$ 243	ND
PPAR $\gamma^{cre}$	72 $\pm$ 5.3 (12)	107 $\pm$ 9.9 (10)	152 $\pm$ 8.1	126 $\pm$ 10.2	456 $\pm$ 83	6,456 $\pm$ 576	2,864 $\pm$ 777	ND
$\beta\gamma$ KO	76 $\pm$ 6.0 (8)	101 $\pm$ 10.9 (10)	137 $\pm$ 6.3	131 $\pm$ 9.3	455 $\pm$ 85	5,583 $\pm$ 421	2,555 $\pm$ 689	ND

<sup>a</sup> Values given are means  $\pm$  standard errors of the means. ND, not done.

<sup>b</sup> n, number of mice.

PPARG in non- $\beta$  cells of  $\beta\gamma$ KO mice. Conversely,  $\beta$  cells from  $\beta\gamma$ KO mice yield no detectable nonrecombined PPAR $\gamma$  transcripts.

Islets from  $\beta\gamma$ KO mice were approximately twice as large as those from PPAR $\gamma^{fl/fl}$  mice on a chow diet (Fig. 3A and B). Morphometric analysis and immunostaining for insulin revealed that the differences in islet sizes were largely accounted for by  $\beta$ -cell hyperplasia. Consistent with these results, BrdU incorporation was proportionately greater in  $\beta\gamma$ KO islets than in PPAR $\gamma^{fl/fl}$  islets; this finding is also consistent with the notion that an increase in cell number rather than cell size contributes to the discrepancy in islet size (Fig. 3C). Insulin content was significantly greater in  $\beta\gamma$ KO islets (3.45  $\pm$  0.28 ng of insulin/ng of DNA) than in PPAR $\gamma^{fl/fl}$  islets (1.75  $\pm$  0.13 ng of insulin/ng of DNA) ( $P = 0.001$ ).

Surprisingly, when the mice were placed on a high-fat diet, quite a different effect was seen. Islets from PPAR $\gamma^{fl/fl}$  mice underwent the expected hyperplastic response that typifies the obese, insulin-resistant state, increasing in mass 8.3-fold. Islets from obese  $\beta\gamma$ KO mice, on the other hand, showed only a 2.1-fold increase in  $\beta$ -cell mass from baseline. Thus, PPAR $\gamma$  is required for the normal expansion of islet mass seen in obesity, demonstrating an unexpected and exciting new role for this receptor in  $\beta$ -cell biology.

**Metabolic function in  $\beta\gamma$ KO mice.** The islet hyperplasia in chow-fed  $\beta\gamma$ KO mice led us to examine whether there were corresponding metabolic alterations in these animals. Male  $\beta\gamma$ KO mice, both fasting and fed, had normal glucose and insulin levels in their sera compared to levels in the three control genotypes, PPAR $\gamma^{fl/fl}$ , PPAR $\gamma^{+/+}$ , and PPAR $\gamma^{cre}$  (Table 1), as well as normal body weight (Fig. 4A). Switching mice to a high-fat diet for up to 55 weeks resulted in impaired glucose tolerance in all genotypes, with no differences in glucose or insulin levels noted between  $\beta\gamma$ KO mice and controls. We then performed glucose and insulin tolerance testing to attempt to unmask a diabetic diathesis. In both chow-fed animals and mice fed a high-fat diet for 55 weeks, no significant differences were seen in glucose excursions after intraperitoneal glucose (Fig. 4B and C) or insulin administration or in glucose-stimulated acute-phase insulin secretion (data not shown). For all of these parameters, trends among female mice did not differ from those seen among males (data not shown).

We next assessed the effect of a PPAR $\gamma$  agonist on insulin secretion from isolated islets. Troglitazone (10  $\mu$ M) significantly improved insulin secretion from PPAR $\gamma^{fl/fl}$  islets in the presence and absence of glucose, consistent with previous reports (45). In contrast, troglitazone had no effect on  $\beta\gamma$ KO

islets (Fig. 5A). This result demonstrates that PPAR $\gamma$  is the sole target of troglitazone in  $\beta$  cells (at least with respect to effects on insulin secretion), and the knockout is likely to be virtually complete in those cells that can synthesize and secrete insulin. More importantly, these results suggested that TZDs might exert their antidiabetic actions, in part, through PPAR $\gamma$  in  $\beta$  cells. In order to definitively address that possibility, we took male PPAR $\gamma^{fl/fl}$  and  $\beta\gamma$ KO mice and placed them on a high-fat diet for 10 weeks. The mice were then treated with either rosiglitazone (3 mg/kg of body weight/day) or vehicle for 3 weeks. For fasting mice, insulin levels in sera obtained after the drug treatment period were higher than those found immediately preceding drug administration in vehicle-treated mice, demonstrating that insulin resistance was still developing (Fig. 5B). Rosiglitazone, however, eliminated this rise in serum insulin levels equally in PPAR $\gamma^{fl/fl}$  and  $\beta\gamma$ KO mice, thus demonstrating that TZD action within  $\beta$  cells does not significantly contribute to the antidiabetic effects of these drugs.

## DISCUSSION

Despite intense investigation, we have a very incomplete understanding of the specific roles played by PPAR $\gamma$  in many of the tissues where it is expressed. The identification of the pancreatic  $\beta$  cell as a site of PPAR $\gamma$  expression, combined with data demonstrating the effects of TZD administration on islet architecture and function, suggested to us that PPAR $\gamma$  could be important in the biology of the  $\beta$ -cell. We have critically analyzed this issue by using Cre-lox technology to target PPAR $\gamma$  in a  $\beta$ -cell-specific manner. Counter to expectations, we could detect no significant metabolic abnormality in  $\beta\gamma$ KO mice relative to the metabolism of PPAR $\gamma^{fl/fl}$ , PPAR $\gamma^{+/+}$ , or PPAR $\gamma^{cre}$  controls. Our analysis included measurements of insulin and glucose in fasting and fed mice on both the lean (chow) and insulin-resistant (high-fat) diets. Glucose and insulin tolerance testing and levels of acute-phase insulin secretion similarly revealed no differences between  $\beta\gamma$ KO and control mice. Although it is not possible to confirm that we have deleted PPAR $\gamma$  expression in every  $\beta$  cell, several lines of evidence indicate that we have eliminated its expression in the vast majority of cells. First, RT-PCR of  $\beta$  cells from the dispersed islets of  $\beta\gamma$ KO mice reveals the absence of wild-type PPAR $\gamma$  mRNA. Second, PCR of whole islets (which contain numerous non- $\beta$  cells, such as  $\alpha$  and  $\delta$  cells, endothelial cells, etc.) reveals a significant reduction in the expression of the floxed allele, while the 400-bp PCR product indicating the presence of the deleted allele is present only in  $\beta\gamma$ KO islets.

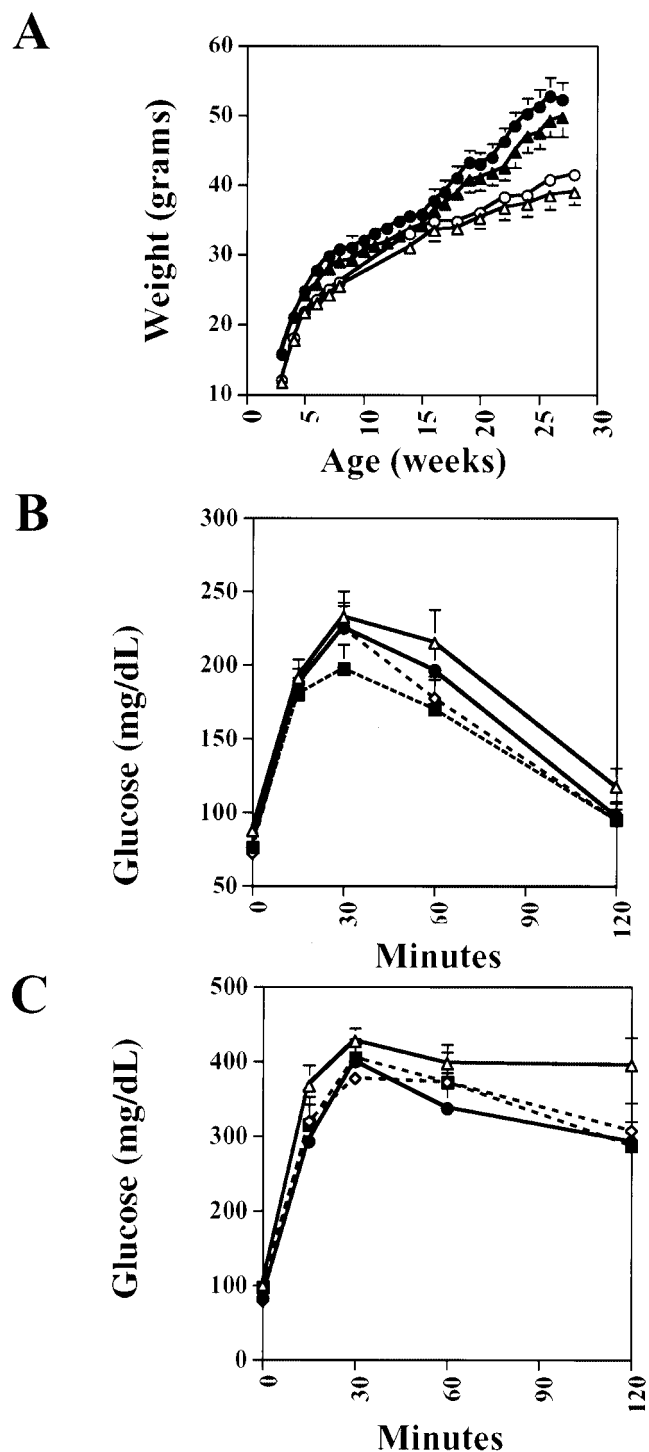


FIG. 4. Metabolic characteristics of male PPAR $\gamma^{fl/fl}$  and  $\beta\gamma$ KO mice. (A) Body weights of PPAR $\gamma^{fl/fl}$  (triangles) and  $\beta\gamma$ KO (circles) mice on the chow (open symbols) and high-fat (filled symbols) diets. (B) Glucose tolerance tests of male mice on chow (16 weeks of age). Phenotypes (numbers of mice) are as follows: PPAR $\gamma^{+/+}$  ( $n = 14$ ), PPAR $\gamma^{cre}$  ( $n = 13$ ), PPAR $\gamma^{fl/fl}$  ( $n = 11$ ), and  $\beta\gamma$ KO ( $n = 16$ ). (C) Glucose tolerance tests of male mice on the high-fat diet (44 weeks of age). Phenotypes (numbers of mice) are as follows: PPAR $\gamma^{+/+}$  ( $n = 6$ ), PPAR $\gamma^{cre}$  ( $n = 10$ ), PPAR $\gamma^{fl/fl}$  ( $n = 15$ ), and  $\beta\gamma$ KO ( $n = 10$ ).

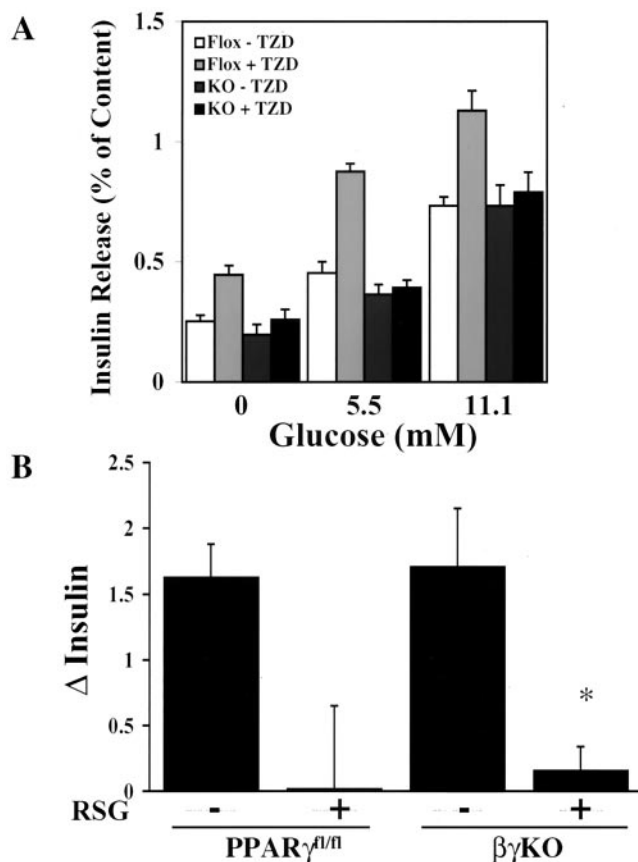


FIG. 5. Effect of TZD drugs on insulin secretion and glucose homeostasis in vivo and in vitro. (A) Isolated islets from PPAR $\gamma^{fl/fl}$  (Flox) and  $\beta\gamma$ KO (KO) mice were cultured overnight in 10  $\mu$ M troglitazone (a TZD drug) or vehicle prior to exposure to glucose at the indicated concentrations. Insulin release is expressed as a percentage of the total content. \*,  $P < 0.05$ ; number of mice for all conditions, 4. (B) Male PPAR $\gamma^{fl/fl}$  and  $\beta\gamma$ KO mice were fed a high-fat diet for 10 weeks, followed by 3 weeks of rosiglitazone (3 mg/kg/day) or vehicle. Serum insulin levels of fasting mice were measured before and after drug administration, and the net changes ( $\Delta$ ) over the treatment period are depicted. Results are means  $\pm$  standard errors of the means. For each group, the number of mice was 8. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; RSG, rosiglitazone.

Third, the effect of troglitazone on insulin secretion is totally eliminated in the  $\beta\gamma$ KO islets. Fourth, in other studies, the expression of the RIP-Cre transgene has been demonstrated to be virtually completely penetrant (24, 25).

The absence of metabolic consequences in  $\beta\gamma$ KO mice does not exclude the  $\beta$  cell as an antidiabetic target of TZD drugs. To assess this possibility, we measured insulin secretion after TZD administration from isolated whole islets of control or  $\beta\gamma$ KO mice. In control islets, troglitazone caused a roughly twofold induction of insulin secretion, even in the absence of glucose. This effect was totally eliminated in  $\beta\gamma$ KO islets. When these studies were extended to the intact mouse, however, we found that the improved glucose homeostasis associated with TZD administration was preserved in  $\beta\gamma$ KO animals. This finding demonstrates that PPAR $\gamma$  in  $\beta$  cells is unlikely to play a major role in the therapeutic response to TZDs. Even if there is a slight improvement in insulin secretion after TZD administration in vivo, this effect is likely masked by the periph-

eral insulin sensitization that dominates the clinical response to TZDs.

The most striking difference between control mice and  $\beta\gamma$ KO mice was islet mass. Loss of PPAR $\gamma$  is associated with a hyperplastic response in the targeted  $\beta$  cells. PPAR $\gamma$  is known to regulate growth in a variety of cell types, including preadipocytes, myeloid leukemia cells, and epithelial tumor lines derived from breast, colon, and prostate. The fact that  $\beta\gamma$ KO islets do not proliferate indefinitely suggests that other growth-regulating pathways eventually come into play, which may explain why tumor formation in the islet tissue of  $\beta\gamma$ KO mice was not observed.

$\beta$ -Cell hyperplasia is most commonly seen as a response to obesity and insulin resistance; it may be caused by a circulating growth factor or factors (14). Despite several theories, no specific factor has been definitively identified. Recent evidence has suggested that insulin itself might be important as a mediator of  $\beta$ -cell hyperplasia (1, 26). All the known components of the insulin signaling pathway are present in  $\beta$  cells (16) (reviewed in reference 23). Loss of insulin receptor substrate 1 (IRS-1) results in hyperplastic, but dysfunctional, islets (3, 27, 39), while loss of IRS-2 results in diabetes without compensatory  $\beta$ -cell hyperplasia (44). The overexpression of a constitutively active form of Akt increases  $\beta$ -cell mass in transgenic mice (7, 42). Furthermore, loss of  $\beta$ -cell insulin receptors ( $\beta$ IRKO) is associated with reduced  $\beta$ -cell hyperplasia during normal aging (24). Crossing  $\beta$ IRKO mice to animals with hepatic insulin resistance (due to the inactivation of insulin receptors in their livers) results in progeny that are insulin resistant but which do not appropriately expand their  $\beta$ -cell mass (R. N. Kulkarni, unpublished data). Given the obvious defect in  $\beta$ -cell hyperplasia after high-fat feeding in  $\beta\gamma$ KO mice, it is tempting to speculate that the lack of PPAR $\gamma$  may cause local insulin resistance within  $\beta$  cells.

Other mechanisms may also contribute to our findings, of course. For example, excess lipid accumulation in the islet has been associated with the lipoapoptosis of  $\beta$  cells (43). TZD treatment of islets from Zucker fatty rats causes reductions in cellular triglyceride levels as well as reduced lipoapoptosis (17, 37). It is possible, therefore, that for  $\beta\gamma$ KO animals on the high-fat diet, the smaller islet mass than that of controls results from enhanced apoptosis in the setting of reduced PPAR $\gamma$  activity.

One of the more intriguing aspects of our study is the discordance between islet mass and function, especially for mice in the obese, insulin-resistant state. Specifically, the fact that glucose and insulin levels are identical in  $\beta\gamma$ KO and PPAR $\gamma^{fl/fl}$  mice on a high-fat diet implies that there must be a compensatory gain in  $\beta$ -cell function in the  $\beta\gamma$ KO mice. This observation is supported by the elevated insulin content on a per cell basis that was seen in  $\beta\gamma$ KO islets (at least in the chow-fed mice). PPAR $\gamma$  can thus be identified as a factor that couples peripheral insulin resistance to changes in  $\beta$ -cell proliferation, perhaps by promoting lipid deposition in the islet. In this scenario, removal of PPAR $\gamma$  makes the  $\beta$  cell work more efficiently. However, obese  $\beta\gamma$ KO mice were still glucose intolerant, indicating that the absence of PPAR $\gamma$  cannot compensate fully for the  $\beta$ -cell dysfunction seen in states of peripheral insulin resistance.

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