JCI The Journal of Clinical Investigation

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J Clin Invest. 2000;105(3):287-292. https://doi.org/10.1172/JCI8538.

Article

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Improved insulin-sensitivity in mice heterozygous for PPAR-γ deficiency

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Received for publication September 23, 1999, and accepted in revised form December 22, 1999.

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J. Clin. Invest. **105**:287–292 (2000).

Introduction

Peroxisome proliferator–activated receptor-γ (PPAR-γ) belongs to a subfamily of nuclear receptors involved in the control of various aspects of lipid metabolism (1). Adipogenesis (2) and other cellular processes of lipid accumulation (3) are stimulated by PPAR-γ through the induction of genes mediating fatty acid metabolism (4–6). In fact, PPAR-γ is induced before the transcriptional activation of most adipose-specific genes. In addition, it plays a critical role in proper placental vascularization, myocardial health, and embryonic development (7). PPAR-γ belongs to a class of nuclear hormone receptors that execute their transcriptional functions as heterodimers with the retinoid-X receptor (RXR) (8–10). The PPAR-γ–RXR heterodimer, in turn, binds to a *cis*-acting sequence (a peroxisome proliferator response element) on DNA to initiate transcription (11).

The ligands for PPAR-γ are diverse and include the natural eicosanoid 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (12, 13) and oxidized LDL particles such as 9- and 13-HODE (14), and the synthetic thiazolidinedione (TZD) compounds (15). TZDs are a new class of insulin-sensitizing drugs used in the treatment of type 2 diabetes. They have been shown to improve insulin sensitivity, glucose tolerance, and the lipidemic profile in type 2 diabetic patients (16, 17) as well as in obese nondiabetic subjects (18). Similar findings have been demonstrated in a number of genetic and nongenetic animal models of diabetes and insulin resistance (19–21).

Despite the wide therapeutic use of TZDs, the physiological roles of PPAR-γ receptors in glucose homeostasis are incompletely understood. To gain further insight into this subject, we studied glucose metabolism in mice heterozygous for PPAR-γ (*PPAR-γ*$^{+/-}$) (7). This approach was chosen due to the nonviability of homozygous PPAR-γ–null animals. Our assumption was that a 50% reduction in the dosage of the receptor may elicit detectable alterations in physiological pathways influenced by PPAR-γ.

We report here that mice carrying a single copy of the *PPAR-γ* gene display greater insulin sensitivity than do their WT age-matched counterparts. Our observations, combined with the established insulin-sensitizing effects of PPAR-γ–activating TZDs, suggest a novel model for the role of PPAR-γ signaling in the etiology of insulin resistance.

Methods

Animals. Mice carrying the *PPAR-γ*–null allele are described elsewhere (7). Genotypes were determined by PCR of tail DNA (7). Animals used in our physiological

Table 1
Basal values

	WT (*n*)	*PPAR-γ*$^{+/-}$ (*n*)
Weight (g)[A]	35.0 ± 1.4 (19)	34.8 ± 1.0 (32)
Epididymal fat-pad weight (g)[B]	1.04 ± 0.09 (13)	0.90 ± 0.08 (26)
Basal glucose (mg/dL)[A]	212 ± 11 (18)	207 ± 7 (30)
Basal insulin (ng/mL)[A]	1.4 ± 0.5 (18)	1.0 ± 0.4 (30)
Basal FFA (mEq/L)[C]	0.71 ± 0.09 (12)	0.90 ± 0.08 (22)

[A]Value measured before OGTT. [B]Value measured at the end of the glucose clamp experiment. [C]Value measured before the glucose clamp experiment.

studies were age-matched WT (*PPAR-γ*$^{+/+}$) and heterozygous (*PPAR-γ*$^{+/-}$) male offspring produced by 4 consecutive backcrosses onto a C57BL/6J strain background (allelic C57BL/6J to 129/SvJae ratio, 15:1). Mice were housed under controlled light (12 hours light/12 hours dark) and temperature conditions, and had free access to food and water. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Animal Subjects Committee of the University of California–San Diego.

Evaluation of mice. Fasting blood samples were taken from the same cohort of mice at 2, 3, 4, and 5 months of age. Animals were sedated with methoxyflurane, and 200 μL of blood was collected by retro-orbital bleed for glucose and insulin measurements.

OGTT. Another group of mice was subjected to an oral glucose tolerance test (OGTT) at approximately 8 months of age. Animals were fasted for 6 hours, and a basal blood sample (225 μL) was collected from the tip of the tail (t = 0 minutes). This was achieved by nicking the tail tip with a scalpel blade, and gently stripping the tail and collecting the blood in a heparinized capillary tube. The mice were then gavaged with dextrose (1.5 g/kg), and additional blood samples were collected at 15, 30, and 60 minutes. All blood samples were spun and the separated plasma was immediately measured for glucose using a Clinical 2300 STAT Glucose & Lactate Analyzer from YSI Inc. (Yellow Springs, Ohio, USA). The remaining plasma was frozen for later determination of insulin levels.

Submaximal hyperinsulinemic, euglycemic glucose clamp. Approximately 2 weeks after the OGTT, each mouse was implanted with 2 catheters (Micro-Renathane MRE 025, 0.025-inch outer diameter, 0.012-inch inner diameter; Braintree Scientific Inc., Braintree, Massachusetts, USA) into the right jugular vein. The anesthetic cocktail consisted of ketamine hydrochloride (80 mg/kg; Fort Dodge Animal Health, Fort Dodge, Iowa, USA), acepromazine maleate (0.5 mg/kg; Butler Co,. Columbus, Ohio, USA), and xylazine (1.6 mg/kg; Butler Co.) given intraperitoneally. Catheters were tunneled subcutaneously, exteriorized at the back of the neck, and filled with heparinized saline. Animals were given 1 mL of saline each subcutaneously and were left to recover for 12 hours on a heating pad.

Four days after surgery, glucose turnover was measured before and during a glucose clamp (the latter is a measure of insulin sensitivity). Each animal was fasted for 5 hours and then placed in a restrainer to which it was accustomed. The glucose clamp was begun with a constant infusion (0.5 μCi/h, 0.1 mL/h) of [3-^{3}H]D-glucose (NEN Life Science Products Inc., Boston, Massachusetts, USA) into a single jugular catheter. Tracer glucose was diluted to 5 μCi/mL in saline containing 100 mg/dL unlabeled D-glucose (Mallinkrodt Inc., Paris, Kentucky, USA) as carrier and 200 mg/dL sodium benzoate (Maliinkrodt) as preservative. One hour after the start of tracer infusion at –10 minutes, a blood sample (225 μL) was collected from the tail tip in heparinized capillary tubes for determination of glucose specific activity and concentration of plasma glucose, insulin, and FFAs. At 0 minutes, regular human insulin (Novolin R; Novo Nordisk Pharmaceutical Industries Inc., Clayton, North Carolina, USA) was infused at 4 mU/kg per minute into the same catheter used for tracer infusion. The insulin infusate was diluted with saline containing 0.1% BSA (Sigma, St. Louis, Missouri, USA). Blood samples (5 μL) were drawn at 10-minute intervals for the immediate determination of blood glucose using a B-Glucose Analyzer from HemoCue Inc. (Mission Viejo, California, USA). Based on these values, 50% dextrose (Abbott Laboratories, Chicago, Illinois, USA) was variably infused into the other jugular catheter to maintain the plasma glucose concentration at approximately 150 mg/dL. Steady state (stable plasma glucose concentration and exogenous glucose infusion rate) was generally achieved within 90–120 minutes, at which time a blood sample (225 μL) was collected for determination of glucose specific activity and concentrations of plasma glucose and insulin. Each animal was then

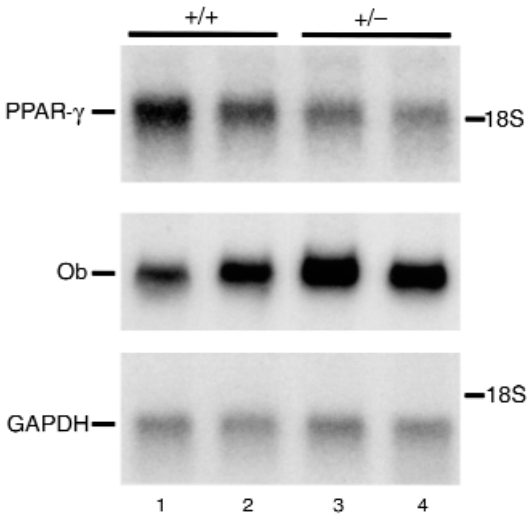

Figure 1
Expression levels of PPAR-γ and Ob in white fat of WT and *PPAR-γ*$^{+/-}$ mice. RNA prepared from the epididymal fat pads of 5-month-old WT mice and *PPAR-γ*$^{+/-}$ littermates was subjected to Northern blot analysis with probes for PPAR-γ, Ob (leptin), and GAPDH.

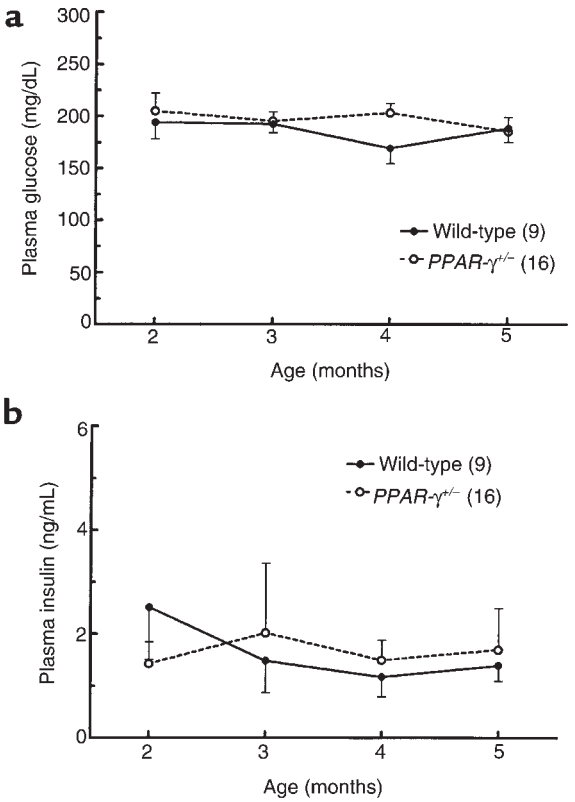

Figure 2
Long-term glucose and insulin concentrations. Fasting glucose (**a**) and insulin (**b**) concentrations of *PPAR-γ*$^{+/-}$ and WT mice taken at 1-month intervals, from 2 months to 5 months of age.

promptly euthanized with pentobarbital and the epididymal fat pads were harvested.

Assays. Insulin was measured using a radioimmunoassay kit (Sensitive Rat Insulin Assay; Linco Research Inc., St. Charles, Missouri, USA). Plasma glucose specific activity was measured after deproteinization with barium hydroxide and zinc sulfate (22).

Calculations. Hepatic glucose production and glucose disposal rate were calculated for the basal period and the steady-state portion of the glucose clamp using the Steele equation for steady-state conditions (23). Values are presented as mean ± SEM. Statistical analysis was performed using 2-way ANOVA for unbalanced data. Significance was assumed at $P < 0.05$.

Results

Animals. Midgestation lethality of PPAR-γ–null embryos (7) prompted us to perform in vivo studies in PPAR-γ heterozygous mice. These animals are fully developed, fertile, and apparently healthy. Due to established effects of sporadic genetic variations between different mouse strains on susceptibility to metabolic disorders, experiments were conducted on animals that were backcrossed for 4 consecutive generations against a C57BL/6J background. The control group was comprised of WT siblings of the heterozygous mice.

As seen in the Northern blots presented in Figure 1, white adipose tissue from *PPAR-γ*$^{+/-}$ mice displayed the predicted decrease in PPAR-γ mRNA expression. Densitometric scanning revealed that PPAR-γ mRNA expression was 170 and 90 (densitometric units) in the WT and *PPAR-γ*$^{+/-}$ mice, respectively. Interestingly, leptin mRNA expression was increased in the *PPAR-γ*$^{+/-}$ animals.

Evaluation of mice. As seen in Figure 2, the fasting plasma glucose and insulin concentrations in the *PPAR-γ*$^{+/-}$ mice were comparable to those in WT mice, and did not change from 2 months to 5 months of age. The weights of the WT mice (35.0 ± 1.4 g) and the *PPAR-γ*$^{+/-}$ mice (34.8 ± 1.0 g) were not different at 8 months of age (see Table 1), nor was there any significant difference in basal FFA levels between the 2 groups (WT, 0.71 ± 0.09 mEq/L; *PPAR-γ*$^{+/-}$, 0.90 ± 0.08 mEq/L; Table 1) when measured just before the glucose clamp experiments. Additionally, the weights of epididymal fat pads harvested after the glucose clamp experiments were comparable between the 2 groups (WT, 1.04 ± 0.15 g; *PPAR-γ*$^{+/-}$, 0.90 ± 0.10 g; Table 1).

OGTT. Due to the absence of statistically significant differences in basal fasting glucose and insulin levels between WT and *PPAR-γ*$^{+/-}$ mice, we next asked whether a 50% reduction in PPAR-γ levels elicits more subtle changes in insulin sensitivity. To this end we

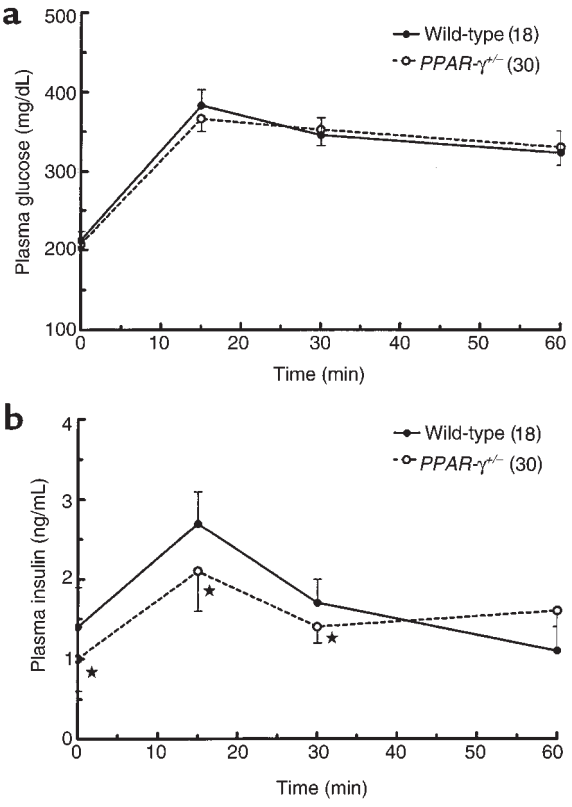

Figure 3
OGTT. Glucose (**a**) and insulin (**b**) concentrations of *PPAR-γ*$^{+/-}$ mice and WT mice that underwent an OGTT (1.5 g/kg) at 8 months of age. *Significantly different from WT mice when values from time-points of 0, 15, and 30 minutes were combined ($P < 0.05$).

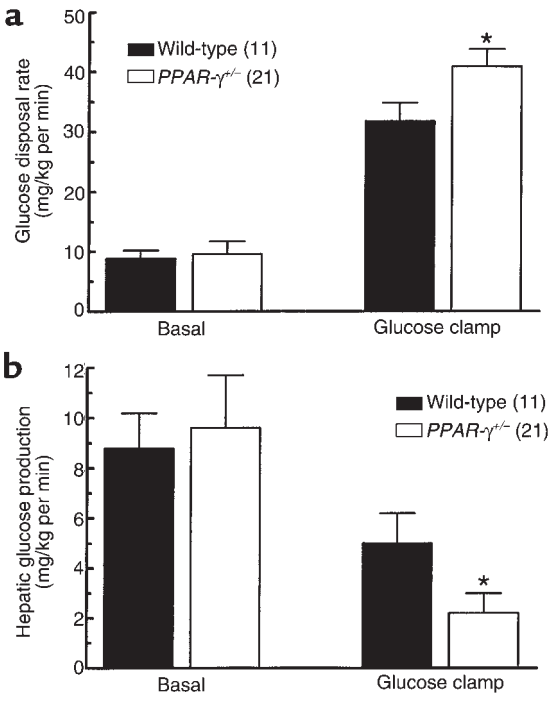

Figure 4
Glucose turnover during glucose clamp experiments. Tracer-determined glucose disposal rate (**a**) and hepatic glucose production (**b**) during the steady-state portion of the hyperinsulinemic, euglycemic glucose clamp experiment for 8-month-old *PPAR-γ*$^{+/-}$ mice and WT mice. *Significantly different from WT mice ($P < 0.05$).

first performed OGTTs on the 8-month-old mice. At the time of testing, levels of basal glucose (WT, 212 ± 11 mg/dL; *PPAR-γ*$^{+/-}$, 207 ± 7 mg/dL) and insulin (WT, 1.4 ± 0.5 ng/mL; *PPAR-γ*$^{+/-}$, 1.0 ± 0.4 ng/mL) in the WT and *PPAR-γ*$^{+/-}$ mice were comparable. As seen in Figure 3, plasma glucose excursions were essentially identical between the 2 groups during the OGTT, demonstrating that the *PPAR-γ*$^{+/-}$ mice were glucose tolerant. However, plasma insulin concentrations in the WT group were greater than in the *PPAR-γ*$^{+/-}$ group when the values from the timepoints of 0, 15, and 30 minutes were combined ($P < 0.05$), suggesting that the *PPAR-γ*$^{+/-}$ mice have increased insulin sensitivity.

Hyperinsulinemic, euglycemic glucose clamps. To directly address a potential alteration in the insulin sensitivity of *PPAR-γ* heterozygotes, we next subjected both groups of mice to hyperinsulinemic, euglycemic glucose clamps. As seen in Figure 4, tracer-derived basal glucose turnover in the *PPAR-γ*$^{+/-}$ mice (9.6 ± 2.1 mg/kg per minute) was not different from that in WT mice (8.8 ± 1.4 mg/kg per minute). During the clamp experiments, the plasma glucose levels of the *PPAR-γ*$^{+/-}$ mice and WT mice were clamped at 143 ± 5 mg/dL and 152 ± 9 mg/dL, respectively ($P < 0.05$). The exogenous glucose infusion rate required to maintain euglycemia during the steady-state portion of the clamp was 38.7 ± 3.1 mg/kg per minute for the *PPAR-γ*$^{+/-}$ mice, and only 26.7 ± 4.9 mg/kg per minute for the WT mice (data not shown). The insulin-induced increase in glucose disposal rate (Figure 4) in the *PPAR-γ*$^{+/-}$ mice (from 9.6 ± 2.9 mg/kg per minute to 40.9 ± 3.1 mg/kg per minute) was significantly greater (~30%) than in the WT mice (from 8.8 ± 1.4 mg/kg per minute to 31.8 ± 4.9 mg/kg per minute; $P < 0.05$). Likewise, the insulin-induced suppression of hepatic glucose production was significantly greater in the *PPAR-γ*$^{+/-}$ mice (from 9.6 ± 2.9 mg/kg per minute to 2.2 ± 0.8 mg/kg per minute) than in the WT mice (from 8.8 ± 1.4 mg/kg per minute to 5.1 ± 1.2 mg/kg per minute; $P < 0.05$). Together, these results indicate that both the liver and peripheral tissues of mice heterozygous for *PPAR-γ* display greater sensitivity to insulin than do the WT controls.

Discussion

TZDs are insulin-sensitizing drugs that improve insulin resistance in human type 2 diabetes (16, 17), obesity (18), and in many experimental animal models of insulin resistance (19–21). These drugs are high-affinity ligands for the nuclear receptor PPAR-γ (15), which regulates target-gene transcription through PPAR response elements (11) as heterodimers with the RXR (8–10). Binding of TZDs to the PPAR-γ half of the heterodimer leads to activation of transcription of responsive genes, including proteins that control lipid and glucose metabolism (4–6). Therefore, it was postulated that reducing the PPAR-γ signal by half using genetic manipulation would lead to a corresponding compromise in insulin sensitivity. Hence, in this study, we examined the role of endogenous PPAR-γ receptors on various aspects of glucose and insulin metabolism by performing OGTTs and hyperinsulinemic glucose clamps in heterozygous PPAR-γ–deficient mice. Surprisingly, our study shows that these mice are significantly more insulin sensitive than are their WT counterparts. Specifically, tracer-determined glucose disposal rates and hepatic glucose production revealed that both the peripheral tissues and the livers of *PPAR-γ*$^{+/-}$ mice are more sensitive to the effects of insulin than are those of WT mice. These results were consistent with the findings during the OGTTs, which showed that the *PPAR-γ*$^{+/-}$ mice were able to maintain glucose levels comparable to those in the WT controls despite significantly lower plasma insulin concentrations. These findings were unexpected and run contrary to what might have been predicted based on the known biological effects and mechanism of action of TZDs. By extension, these results raise the possibility that inhibition of PPAR-γ function could render individuals less susceptible to the development of insulin resistance caused by obesity, type 2 diabetes, aging, or other factors.

How the heterozygotic *PPAR-γ* genotype augments insulin action remains uncertain; however, our studies show that body weight, fat-pad mass, and FFA levels of the *PPAR-γ*$^{+/-}$ animals were comparable to those of their WT littermates. Our findings are consistent with recent work from Kubota et al. (24). These investigators found that ingestion of a high-fat diet led to insulin resistance

in WT mice, but failed to alter insulin sensitivity in *PPAR-γ*$^{+/-}$ mice. It has been reported that TZDs can decrease leptin mRNA expression in adipose tissue (25), and it is of interest that leptin expression was enhanced in adipose tissue from our *PPAR-γ*$^{+/-}$ mice. Although the *PPAR-γ*$^{+/-}$ mice did not eat less or weigh less than did the WT controls, to the extent that serum leptin levels might be increased, it is possible that leptin could influence insulin sensitivity.

Although our experiments do not provide a molecular mechanism to explain these results, they do suggest a hypothesis that runs counter to the current dogma on PPAR-γ biology, at least with respect to glucose homeostasis. One possibility lies within the nature of PPAR-γ ligands. Because TZDs enhance insulin sensitivity, it has been assumed that PPAR-γ and its natural ligands maintain or enhance insulin sensitivity by ensuring proper expression levels of key glucoregulatory genes. However, an alternative interpretation is that the endogenous receptor, along with its natural ligands, might serve to dampen insulin action, thereby promoting insulin resistance. Teleogically, this could serve to offset exaggerated effects on glucose metabolism caused by unrestrained activation of the differentiation program induced by PPAR-γ stimulation. If this were the case, then decreased expression of PPAR-γ receptors (as seen in our heterozygotic mice) would partially alleviate the dampening effect, leading to heightened insulin sensitivity.

Two different possibilities, consistent with this scenario, could account for the molecular basis of TZD action. The first reevaluates the nature of synthetic PPAR-γ ligands. We tend to think of these compounds as pure activators, when in reality most have been shown to be partial agonists; this makes them in effect also partial antagonists, able to displace putative endogenous full agonists. According to this hypothesis, the insulin-sensitizing effects of TZDs may be due to their ability to partially inhibit the dampening effect of insulin action with a natural ligand (or ligands) working through endogenous PPAR-γ receptors. An alternative hypothesis that is consistent with both the pharmacological data and our new genetic findings is related to the nature of the transcriptional activity of PPAR-γ receptors. It is usually assumed that endogenous PPAR-γ receptors rotate between a transcriptional activator and an inactive mode. However, closely related members of the nuclear receptor superfamily, such as the retinoic acid and thyroid hormone receptors, can — in addition to their transactivation function — mediate active sequence–specific repression when the ligand is absent (26–28). The molecular modules involved in this response are fully conserved in the PPAR-γ receptor, which has been shown to interact with corepressors in its ligand-free state in vitro (29, 30). Thus, it is possible that a component of insulin resistance is mediated by PPAR-γ–induced transcriptional repression, and that insulin sensitivity is restored either by reversing this repression with a TZD partial agonist or by genetically reducing the levels of the repressor protein, i.e., PPAR-γ receptor.

In conclusion, our data indicate that a genetic reduction in *PPAR-γ* gene expression can lead to enhanced insulin sensitivity. This suggests that normally, full PPAR-γ activity may play a role in the development of states of insulin resistance such as type 2 diabetes. The hypotheses outlined above are obviously quite speculative; nevertheless, these ideas can serve as templates for future experimentation into the mechanisms by which PPAR-γ receptors modulate insulin action and glucose homeostasis.

Acknowledgments

We thank Michael C. Nelson for excellent mouse colony management and genotyping. We thank T. Kadowaki for communicating unpublished *PPAR-γ* knockout data. Y. Barak was supported by an EMBO fellowship and by funds from the Charles and Anna Stern Foundation. R.M. Evans is an investigator of the Howard Hughes Medical Institute at the Salk Institute for Biological Studies, and is the March of Dimes chair of molecular and developmental biology. This work was supported in part by grants from the National Institutes of Health (DK-33651 and HD-27183) and the Veterans Administration Research Service, Department of Veterans Affairs.

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