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The Mechanisms by Which Both Heterozygous Peroxisome Proliferator-activated Receptor γ (PPARγ) Deficiency and PPARγ Agonist Improve Insulin Resistance*

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Toshimasa Yamauchi‡, Junji Kamon‡§, Hironori Waki‡§, Koji Murakami¶, Kiyoto Motojima‖, Kajuro Komeda, Tomohiro Ide¶, Naoto Kubota‡, Yasuo Terauchi‡, Kazuyuki Tobe‡, Hiroshi Miki‡, Atsuko Tsuchida**, Yasuo Akanuma‡‡, Ryozo Nagai‡, Satoshi Kimura‡, and Takashi Kadowaki‡§§**

*From the ‡Department of Internal Medicine, Graduate School of Medicine, University of Tokyo, Tokyo 113-8655, ¶Central Research Laboratories, Kyorin Pharmaceutical, Tochigi 329-0114, ‖Department of Biochemistry, Meiji Pharmaceutical University, Tokyo 204-8588, **Division of Laboratory Animal Science, Animal Research Center, Tokyo Medical University, Tokyo 160-8402, and ‡‡Institute for Diabetes Care and Research, Asahi Life Foundation, Tokyo 100-0005, Japan*

Peroxisome proliferator-activated receptor (PPAR) γ is a ligand-activated transcription factor and a member of the nuclear hormone receptor superfamily that is thought to be the master regulator of fat storage; however, the relationship between PPARγ and insulin sensitivity is highly controversial. We show here that supraphysiological activation of PPARγ by PPARγ agonist thiazolidinediones (TZD) markedly increases triglyceride (TG) content of white adipose tissue (WAT), thereby decreasing TG content of liver and muscle, leading to amelioration of insulin resistance at the expense of obesity. Moderate reduction of PPARγ activity by heterozygous PPARγ deficiency decreases TG content of WAT, skeletal muscle, and liver due to increased leptin expression and increase in fatty acid combustion and decrease in lipogenesis, thereby ameliorating high fat diet-induced obesity and insulin resistance. Moreover, although heterozygous PPARγ deficiency and TZD have opposite effects on total WAT mass, heterozygous PPARγ deficiency decreases lipogenesis in WAT, whereas TZD stimulate adipocyte differentiation and apoptosis, thereby both preventing adipocyte hypertrophy, which is associated with alleviation of insulin resistance presumably due to decreases in free fatty acids, and tumor necrosis factor α, and up-regulation of adiponectin, at least in part. We conclude that, although by different mechanisms, both heterozygous PPARγ deficiency and PPARγ agonist improve insulin resistance, which is associated with decreased TG content of muscle/liver and prevention of adipocyte hypertrophy.

Peroxisome proliferator-activated receptor (PPAR)[1] γ is a ligand-activated transcription factor and a member of the nuclear hormone receptor superfamily that functions as a heterodimer with a retinoid X receptor (RXR) (1–5). Agonist-induced activation of PPARγ/RXR is known to increase insulin sensitivity (6, 7). Thiazolidinediones (TZD), which have the ability to directly bind and activate PPARγ (6) and to stimulate adipocyte differentiation (2, 8), are used clinically to reduce insulin resistance and hyperglycemia in type 2 diabetes (1, 2, 4, 5). We and others (9, 10) have reported that heterozygous PPARγ-deficient mice are protected from high fat (HF) diet- or aging-induced adipocyte hypertrophy, obesity, and insulin resistance. Consistent with this, the Pro-12 → Ala polymorphism in human PPARγ2, which moderately reduces the transcriptional activity of PPARγ, has been shown to confer resistance to type 2 diabetes (11–13). This apparent paradox raises the following important unresolved issue (14) which we addressed experimentally in this study. We attempted to explain how insulin resistance could be improved by two opposite PPARγ activity states, supraphysiological activation of PPARγ and moderate reduction. We did so by using heterozygous PPARγ-deficient mice and a pharmacological activator of PPARγ in wild-type mice.

We show here that supraphysiological activation of PPARγ by TZD markedly increases triglyceride (TG) content of white adipose tissue (WAT), thereby decreasing TG content of liver and muscle, leading to amelioration of insulin resistance at the expense of obesity. Moderate reduction of PPARγ activity by heterozygous PPARγ deficiency decreases TG content of WAT, skeletal muscle, and liver due to increased leptin expression and increase in fatty acid combustion and decrease in lipogenesis, thereby ameliorating HF diet-induced obesity and insulin resistance. Moreover, although heterozygous PPARγ deficiency and TZD have opposite effects on total WAT mass, heterozygous PPARγ deficiency decreases lipogenesis in WAT, whereas TZD stimulate adipocyte differentiation and apoptosis, thereby both preventing adipocyte hypertrophy. This results in a decrease in molecules causing insulin resistance such as free fatty acids (FFA) (15) and tumor necrosis factor (TNF) α (16) and

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§ Contributed equally to this work.

§§ To whom correspondence should be addressed: Dept. of Internal Medicine, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Tel.: 81-3-5800-8818; Fax: 81-3-5689-7209; E-mail: kadowaki-3im@h.u-tokyo.ac.jp.

[1] The abbreviations used are: PPARγ, peroxisome proliferator-activated receptor; WAT, white adipose tissue; TG, triglyceride; TZD, thiazolidinediones; RXR, retinoid X receptor; FFA, free fatty acids; TNF, tumor necrosis factor; HC, high carbohydrate; SREBP, sterol regulatory element-binding protein; IRS, insulin receptor substrate; HF, high fat; PI, phosphatidylinositol; BAT, brown adipose tissue.

up-regulation of insulin-sensitizing hormone adiponectin (17), thereby leading to alleviation of insulin resistance. We conclude that, although by different mechanisms, both heterozygous PPARγ deficiency and PPARγ agonist improve insulin resistance, which is associated with decreased TG content of muscle/liver and prevention of adipocyte hypertrophy.

EXPERIMENTAL PROCEDURES

Chemicals—Rosiglitazone was synthesized as described elsewhere (6). Wy-14,643 was purchased from Biomol (Plymouth Meeting, PA). All other materials were from the sources given in Refs. 8 and 9.

Animals, in Vivo Glucose Homeostasis, Assay of Endogenous Serum Leptin Concentrations, and Leptin Sensitivity—Heterozygous PPARγ-deficient mice have been described (9). All other animals were purchased from Nippon CREA Co., Ltd. Six-week-old mice were fed powdered chow according to methods described previously (9). Drugs were given as food admixtures (8, 9), and there was no toxicity observed including liver damage. The area of glucose and insulin curves was calculated by multiplying the cumulative mean height of the glucose values (1 mg ml^{-1} = 1 cm) and insulin values (1 ng ml^{-1} = 1 cm), respectively, by time (60 min = 1 cm) as described in Ref. 7. The results are expressed as the percentage of the value of each controls. The insulin resistance index (7) was calculated from the product of the areas of glucose and insulin $\times 10^{-2}$ in glucose tolerance test (9). The results are expressed as the ratio of the value of each wild-type controls on the high carbohydrate (HC) diet (9). Leptin was assayed with the enzyme-linked immunosorbent assay-based Quantikine M mouse leptin immunoassay kit (R & D Systems) according to the manufacturer's instructions. For leptin sensitivity (9), leptin (PeproTech) was administered to mice as a daily intraperitoneal injection of 10 μg/g body weight/day. Isotonic sodium chloride solution was administered to the controls. Food intake and body weight were measured to assess the effects of leptin administration.

Histological Analysis of Adipose Tissue and Determination of Adipocyte Size—Adipose tissue was removed from each animal, fixed in 10% formaldehyde/phosphate-buffered saline, and maintained at 4 °C until used. Fixed specimens were dehydrated, embedded in tissue-freezing medium (Tissue-Tek OCT compound; Miles), and frozen in dry ice and acetone. WAT was cut into 10-μm sections, and the sections were mounted on silanized slides. The adipose tissue was stained with hematoxylin and eosin. Mature white adipocytes were identified by their characteristic multilocular appearance. Total adipocyte areas were traced manually and analyzed with Win ROOF software (Mitani Co., Ltd., Chiba, Japan). White adipocyte areas were measured in 400 or more cells per mouse in each group according to the methods described previously (8, 9). Sections of adipose tissues from mice treated for 14 days were stained by the terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling technique with a kit (*In Situ* Cell Death Detection Kit, AP; Roche Molecular Biochemicals) to detect apoptotic nuclei as described (8), with slight modifications. The numbers of all nuclei and apoptosis positive-stained nuclei were counted to calculate the ratio to the number of apoptotic nuclei to total number of nuclei.

RNA Preparation, Northern Blot Analysis, RNase Protection Assay, PI3-Kinase Assay, Immunoprecipitation, and Immunoblotting—Total RNA was prepared from tissues with TRIzol (Life Technologies, Inc.) according to the manufacturer's instructions. Total RNA from 5 to 10 mice in each group was pooled, and aliquots were subjected to Northern blot analysis with the probes for rat acyl-CoA oxidase (Dr. T. Hashimoto), mouse CD36, UCP2, and adiponectin cDNA or RNase protection assay to measure mRNAs of TNFα performed using a standard protocol (8, 9, 18, 19). The radioactivity in each band was quantified, and the fold change in each mRNA was calculated after correction for loading differences by measuring the amount of 28 S rRNA. Very low levels (<10%) of adipocyte P2 mRNA were detected in muscle as compared with those in WAT. By contrast, CD36, SCD1, acyl-CoA oxidase, and UCP2 mRNAs were detected in muscle at levels comparable to those in WAT. These findings suggest that the results for muscle tissue essentially represent the results for the muscle cells, although the muscle was contaminated by a small amount (<10%) of inter-myocyte fat (20). The procedures used for PI3-kinase assay, immunoprecipitation, and immunoblotting have been described previously (21). Representative data from one of three independent experiments are shown.

Lipid Metabolism and Measurement of Tissue TG Content—The measurements of $[^{14}C]CO_2$ production from [1-^{14}C]palmitic acid and lipogenesis from [1-^{14}C]acetate were performed using liver, muscle, and WAT slices, as described (18, 22). Liver and muscle homogenates were extracted, and their TG content was determined as described previously (18), with some modifications.

RESULTS

TZD Improve Insulin Resistance At the Expense of Obesity, whereas Heterozygous PPARγ Deficiency Improves Both Insulin Resistance and Obesity—To explain how insulin resistance could be improved by two opposite PPARγ activity states, supraphysiological activation of PPARγ and moderate reduction, we studied the phenotypes of untreated or PPARγ agonist-treated wild-type mice and untreated heterozygous PPARγ-deficient mice. We assessed PPARγ activity *in vivo* by measuring expression levels of lipoprotein lipase (23), fatty-acid translocase (FAT)/CD36 (24), and adipocyte fatty acid-binding protein/adipocyte P2 (25) (Fig. 1*A*), whose promoters contain peroxisome proliferator response element, in WAT, where PPARγ is expressed most predominantly *in vivo*. As expected, rosiglitazone-treated wild-type mice exhibited a significant increase in PPARγ activity as compared with untreated wild-type mice (Fig. 1*A*, *lanes 1* and *2*), whereas untreated heterozygous PPARγ-deficient mice showed a moderate decrease in PPARγ activity (Fig. 1*A*, *lanes 1* and *3*).

Untreated wild-type mice on the HF diet gained significantly more body weight than the mice on the HC diet (data not shown). Administration of rosiglitazone to wild-type mice increased significantly more body weight than vehicle on the HF diet (Fig. 1*B, lanes 1* and *2*). In contrast, heterozygous PPARγ deficiency reduced the increase in body weight on the HF diet (Fig. 1*B, lanes 2* and *3*). Treatment of wild-type mice with rosiglitazone significantly increased WAT mass (Fig. 1*C*, *lanes 1* and *2*), whereas untreated heterozygous PPARγ-deficient mice were protected from HF diet-induced increase in WAT mass (Fig. 1*C*, *lanes 2* and *3*). These data suggested that PPARγ determines the adiposity in proportion to its activity.

Treatment of wild-type mice with rosiglitazone improved hyperglycemia (Fig. 1*D, lanes 1*) and hyperinsulinemia (Fig. 1*E,* lane 1) on the HF diet as compared with untreated wild-type mice (Fig. 1, *D* and *E, lane 2*). Untreated heterozygous PPARγ-deficient mice were also protected from HF diet-induced hyperglycemia (Fig. 1*D, lanes 2* and *3*) and hyperinsulinemia (Fig. 1*E, lanes 2* and *3*). These findings indicate that TZD improve insulin sensitivity at the expense of obesity, whereas moderate reduction of PPARγ activity has potential as anti-obesity and anti-diabetic drugs.

Heterozygous PPARγ Deficiency Exerts Its Anti-obesity and Anti-diabetic Effects in Part through Leptin-dependent Pathways—The rectal temperature was lower in rosiglitazone-treated wild-type mice than that in untreated wild-type mice (Fig. 2*A, lanes 1* and *2*); on the contrary, it was significantly higher in untreated heterozygous PPARγ-deficient mice (Fig. 2*A, lanes 2* and *3*). The serum leptin (26) levels were slightly but not significantly lower in rosiglitazone-treated wild-type mice than those of untreated wild-type mice (Fig. 2*B*, *lanes 1* and *2*), whereas they were significantly higher in untreated heterozygous PPARγ-deficient mice (Fig. 2*B, lanes 2* and *3*). Thus the serum leptin levels were parallel to the rectal temperature. It was also noted that serum leptin levels were negatively correlated with PPARγ activity, suggesting that the serum leptin levels were parallel to the repression of leptin gene transcription by PPARγ/RXR (27). Moreover, leptin sensitivity as assessed by reductions in food intake and body weight change in response to exogenously administered leptin was significantly increased in heterozygous PPARγ-deficient mice as compared with wild-type mice on the HF diet (Fig. 2*C* and *D*, *lanes 3–6*). The degree of change in body weight induced by leptin treatment differed significantly ($p < 0.01$) between wild-type ($-0.67 \pm 0.09\%$) and heterozygous PPARγ-deficient

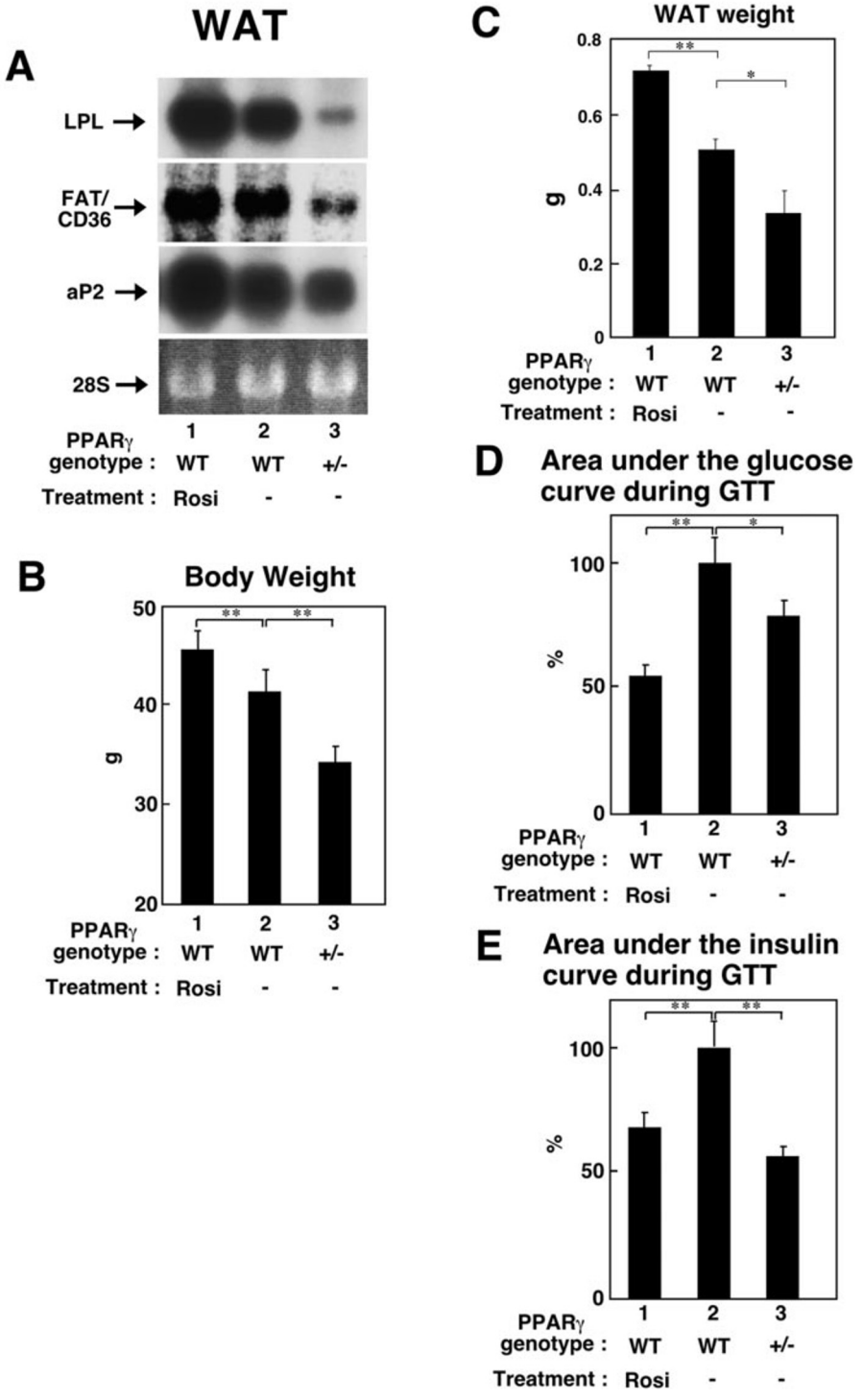

FIG. 1. **TZD improve insulin resistance at the expense of obesity, whereas heterozygous PPARγ deficiency improves both insulin resistance and obesity.** Amounts of the mRNAs of lipoprotein lipase (*LPL*), fatty-acid translocase (*FAT*)/CD36, adipocyte fatty acid binding protein/adipocyte P2 (*aP2*) in white adipose tissue (*A*), body weight (*B*), WAT weight (*C*), the values of area under the glucose curve (*D*), and area under the insulin curve (*E*) during glucose tolerance test (*GTT*) of wild-type (*WT*) and heterozygous PPARγ-deficient mice (+/−) untreated (−) or treated with rosiglitazone (*Rosi*) for 4 weeks (*A–E*) while on the high fat diet are shown. Rosiglitazone was given as a 0.01% food admixture. Each *bar* represents the mean ± S.E. (n = 5–10). *, $p < 0.05$; **, $p < 0.01$.

mice (−1.38 ± 0.11%). These data raised the possibility that increased leptin effects may contribute to the effects of the heterozygous PPARγ deficiency.

Heterozygous PPARγ Deficiency Decreases Lipogenesis in WAT, whereas TZD Stimulate Adipocyte Differentiation and Apoptosis, Thereby Both Preventing Adipocyte Hypertrophy—In WAT from untreated heterozygous PPARγ-deficient mice, expressions of lipoprotein lipase and CD36 were reduced, which may contribute to decreased TG content. In addition, expressions of lipogenic enzymes such as sterol regulatory element-binding protein (SREBP) 1c and SCD (stearoyl-CoA desaturase) 1 were reduced, and lipid synthesis was indeed significantly decreased in WAT from heterozygous PPARγ-deficient mice as compared with that in wild-type mice on the HF diet (Fig. 2*F*). Expression of β_3-adrenergic receptor (Fig. 2*E*, *lanes 2* and *3*) was increased presumably due to their increased leptin effects (Fig. 2, *B–D*) (28) and decreased PPARγ/RXR effects (29), and fatty acid oxidation was increased (data not shown). Decreased lipid synthesis and increased fatty acid oxidation as well as presumably decreased fatty acid influx in heterozygous PPARγ-deficient mice may in concert prevent adipocyte hypertrophy (Fig. 3*A*), and therefore obesity (Fig. 1, *B* and *C*, *lanes 2* and *3*), on the HF diet.

Interestingly, supraphysiological activation of PPARγ significantly reduced the average size of adipocytes under the HF diet (Fig. 3*A*, *lane 1* and *2*) as a result of a marked increase in

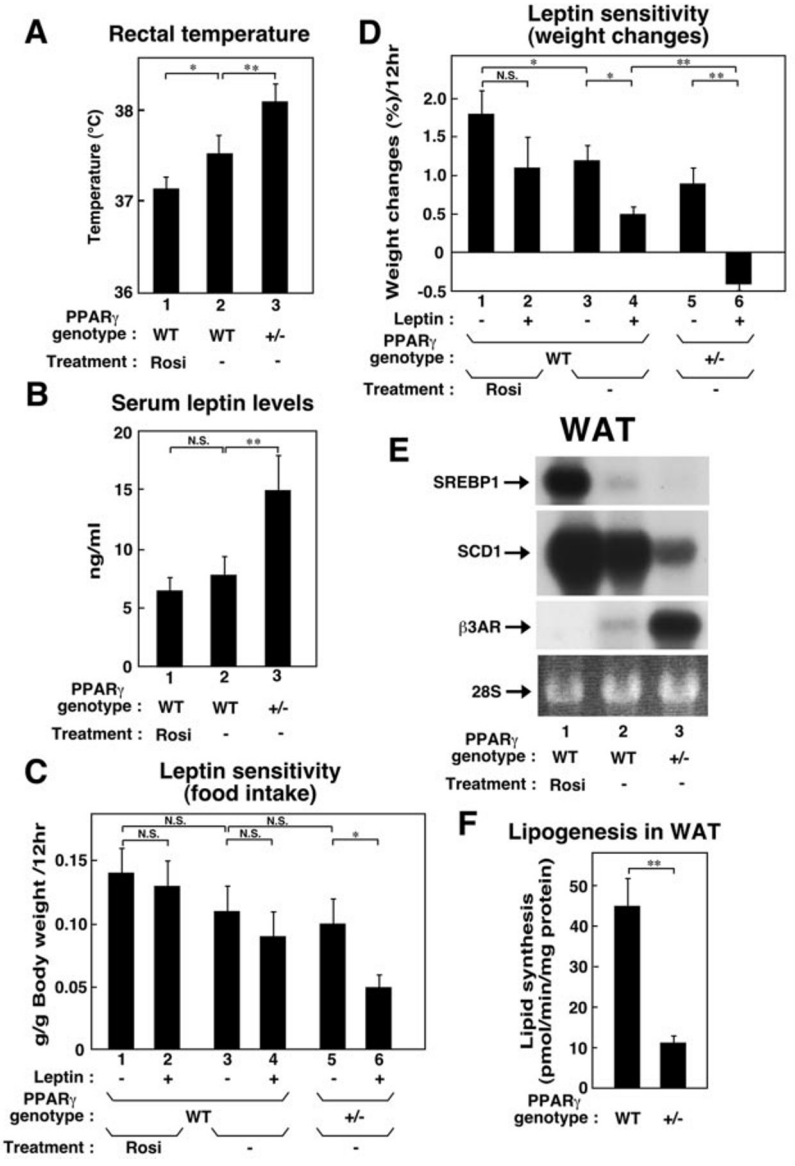

FIG. 2. **Heterozygous PPARγ deficiency improves both insulin resistance and obesity in part through leptin-dependent pathways.** The rectal temperature (*A*), serum leptin levels (*B*), amounts of the mRNAs of SREBP 1, stearoyl Co-A desaturase (*SCD*) 1, and β_3-adrenergic receptor (*AR*) in WAT (*E*), lipid synthesis from [^{14}C]acetate in WAT (*F*), effects of intraperitoneal leptin administration in wild-type (*WT*), and heterozygous PPARγ-deficient mice (+/−) untreated (−) or treated with rosiglitazone (*Rosi*) for 10 days (*B*) or 4 weeks (*A* and *C-F*) while on the HF diet are shown. Mice received an intraperitoneal injection of either leptin (10 μg/g/day) (+) or isotonic sodium chloride solution (−). Food intake/12 h (*left*) and weight changes/12 h (*right*) were measured. Rosiglitazone was given as a 0.01% food admixture. Each *bar* represents the mean ± S.E. (n = 5–10). *, $p < 0.05$; **, $p < 0.01$; *N.S.,* no significant difference; compared with untreated wild-type mice.

the number of newly differentiated small adipocytes and significant decrease in the number of large adipocytes with a concomitant induction of apoptosis of adipocyte (Fig. *3B, lanes 1* and *2,* and Fig. 3*C*) (8). On the other hand, heterozygous PPARγ deficiency appeared to prevent HF diet-induced adipocyte hypertrophy without a significant change in the total number of adipocytes (Fig. 3*B, lanes 2* and *3* and Fig. 3*C*). These data suggest that the heterozygous PPARγ deficiency decreases lipogenesis in WAT, whereas TZD stimulate adipocyte differentiation and apoptosis, thereby both preventing adipocyte hypertrophy.

Adipocyte Hypertrophy Is Associated with Insulin Resistance—We next attempted to experimentally clarify the relationships between adipocyte hypertrophy and insulin resistance. To this end, we induced adipocyte hypertrophy by high fat feeding, leptin receptor deficiency, or agouti overexpression. The size of the adipose cells and the insulin resistance were increased in mice on a HF diet compared with those in mice on a HC diet (Fig. 3*D*). The size of the adipose cells and the insulin resistance of db/db mice were also increased compared with their wild-type controls on both the HC and HF diet (Fig. 3*D*). We obtained essentially similar results by using KKAy mice and their wild-type controls (KK) (Fig. 3*E*). These findings support a close correlation between adipocyte hypertrophy and insulin resistance, even though a cause and effect relationship is again unproven. In this context, protection from adipocyte hypertrophy due to decreased lipid synthesis in WAT from heterozygous PPARγ-deficient mice (Fig. 2*F*) may cause an increase in insulin sensitivity (Fig. 3*F*).

Protection from Adipocyte Hypertrophy May Finally Lead to Alleviation of Insulin Resistance Presumably via a Decrease in Molecules Causing Insulin Resistance and Up-regulation of Insulin-sensitizing Hormones—We tried to clarify the molecular link between adipocyte hypertrophy and insulin resistance. We examined the levels of expression of molecules secreted by WAT that regulate insulin sensitivity under the following four different conditions: HC feeding, HF feeding, HF feeding with heterozygous PPARγ deficiency, and HF feeding with PPARγ agonist. The HF diet significantly increased adipocyte size and at the same time increased the molecules causing insulin resistance, such as FFA and TNFα (Fig. 3, *G* and *H*), and decreased the molecules causing insulin sensitivity, such as adiponectin (Fig. 3*I*), in mice that exhibited insulin resistance as compared with mice on the HC diet (Fig. 1, *D* and *E, lanes 1* and *2*). (Replenishment of adiponectin in KKAy mice on the HF diet partially reverses insulin resistance even at the doses that do not significantly change adipocyte size (17).) In addition, treatment of wild-type mice with the PPARγ agonist rosiglitazone or

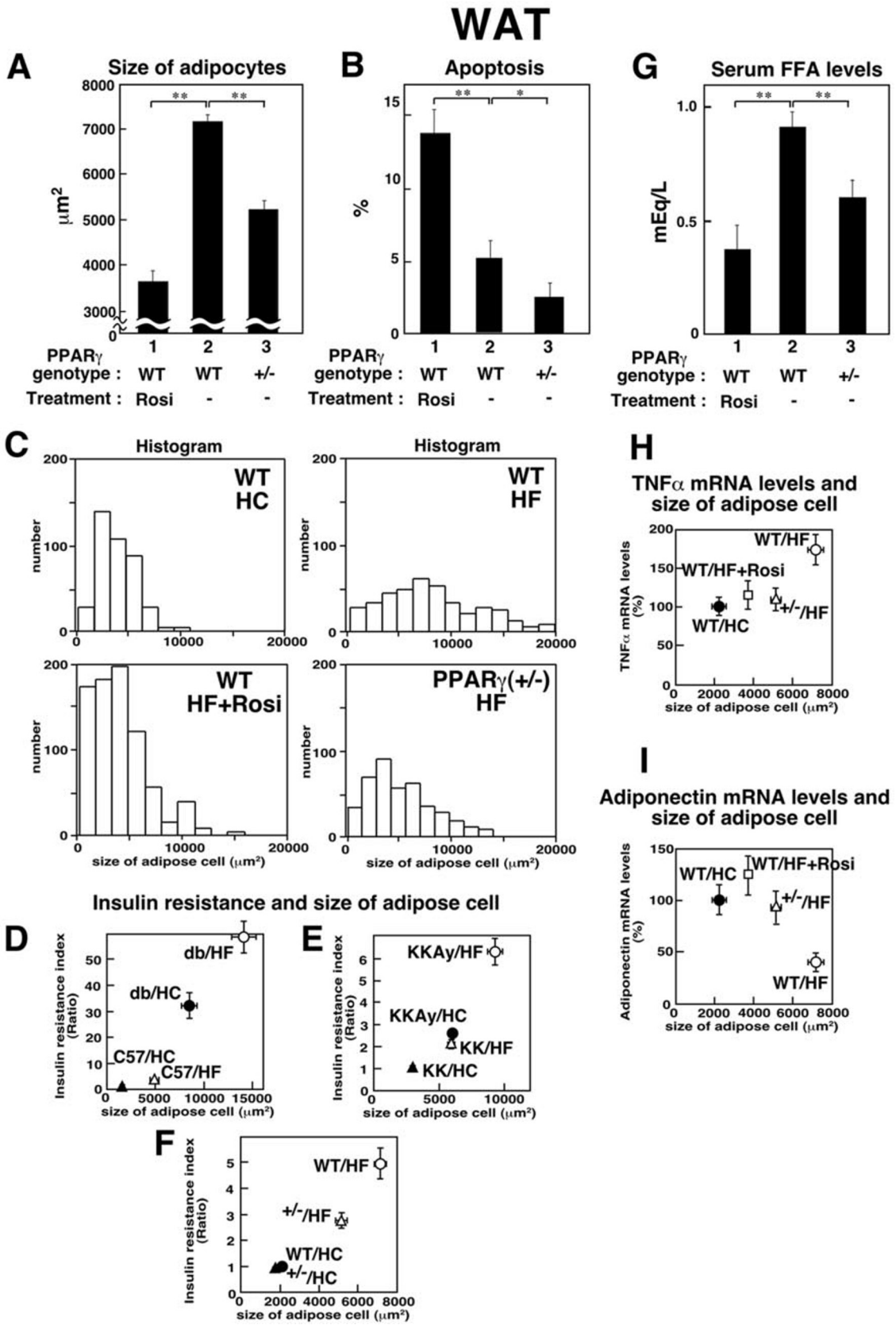

FIG. 3. **Both heterozygous PPARγ deficiency and TZD prevent adipocyte hypertrophy, which finally lead to alleviation of insulin resistance via a decrease in molecules causing insulin resistance and up-regulation of insulin-sensitizing hormone, at least in part.** *A–E,* the average size of adipocytes (*A*), the ratio of apoptotic nuclei (*B*), distribution of adipose cell size (*C*) of epididymal WAT, the relationship between insulin resistance and average size of adipose cells (*D–F*), serum free fatty acid levels (*G*), the relationship between average size of adipose cells and amounts of the mRNAs of TNFα (*H*), or adiponectin (*I*) in WAT in wild-type (*WT*) and heterozygous PPARγ-deficient mice (+/−) (*A-C* and *F-I*) or C57 and db/db (db) mice (*D*) or KK and KKAy mice (*E*) untreated (−) or treated with rosiglitazone (*Rosi*) for 4 weeks while on the HC (*C–F*) or HF diet (*A–I*). Rosiglitazone was given as a 0.01% food admixture. Each *bar* represents the mean ± S.E. (n = 5–10). *, $p < 0.05$; **, $p < 0.01$; compared with untreated wild-type mice.

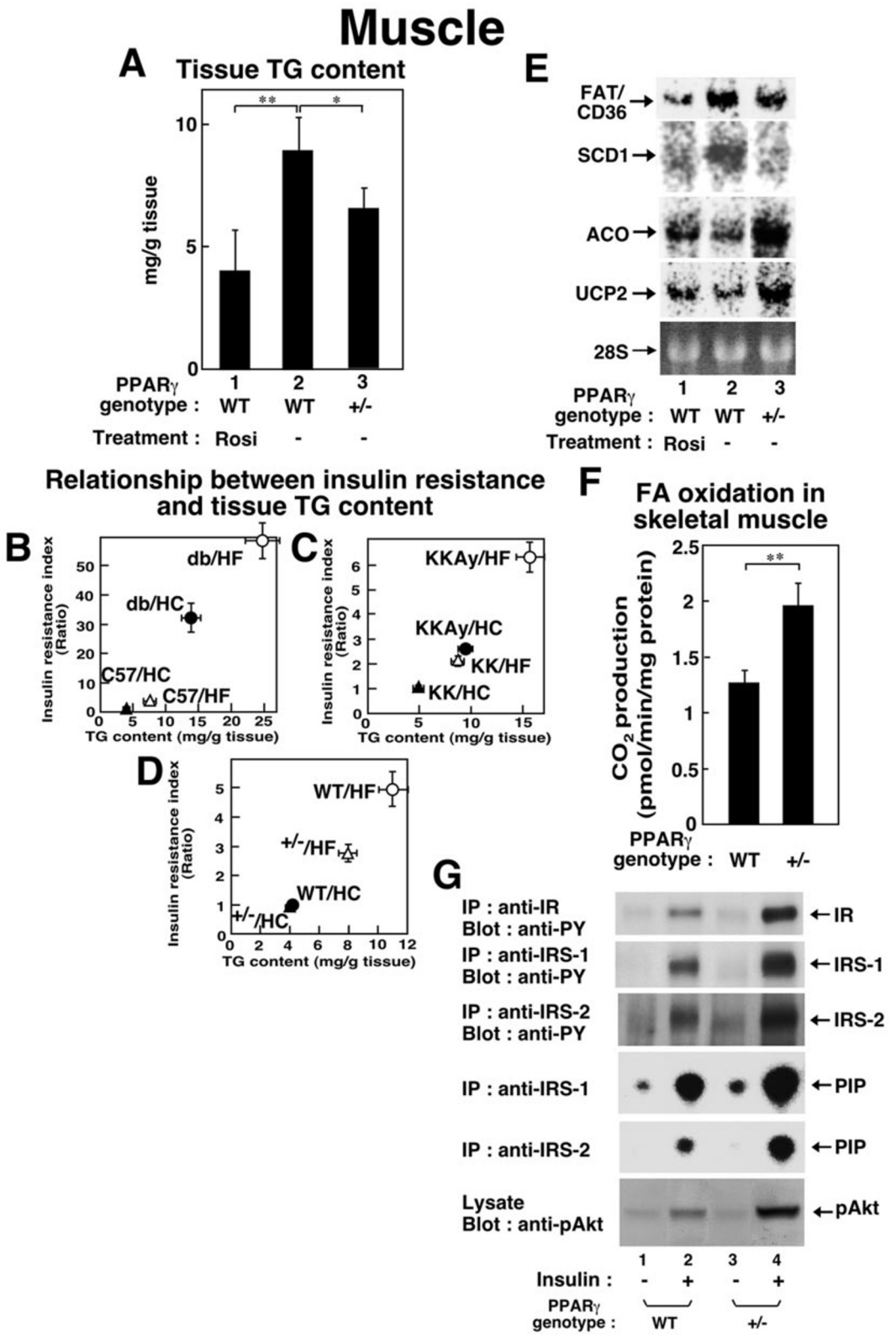

FIG. 4. **TZD indirectly decreases molecules involved in FFA influx into muscle, whereas heterozygous PPARγ deficiency increases fatty acid oxidation and molecules involved in energy dissipation, thereby both decreasing tissue TG content in muscle.** Tissue triglyceride (*TG*) content in skeletal muscle (*A*), the relationship between insulin resistance and tissue TG content in muscle (*B–D*), amounts of the mRNAs of fatty-acid translocase (*FAT*)/CD36, stearoyl Co-A desaturase (*SCD*) 1, acyl-CoA oxidase (*ACO*), and uncoupling protein (*UCP*) 2 (*E*), fatty acid (*FA*) oxidation (*F*), and insulin-induced tyrosine phosphorylation of insulin receptor (*IR*) and IRS-1 and -2, and insulin-stimulated PI3-kinase activity and insulin-induced phosphorylation of Akt (*G*) in muscle of wild-type (*WT*) and heterozygous PPARγ-deficient mice (+/−) (*A* and *D-G*) or C57 and db/db (*db*) mice (*B*) or KK and KKAy mice (*C*) untreated (−) or treated with rosiglitazone (*Rosi*) for 4 weeks while on the HF diet (*A–G*) or HC diet (*B–D*). Rosiglitazone was given as a 0.01% food admixture. Fatty acid oxidation was assessed by the measurements of $[^{14}C]CO_2$ production from [^{14}C]palmitic acid (*F*). Mice were stimulated with or without 1 μg/g body weight of insulin for 2 min. Lysates were immunoprecipitated (*IP*) with the antibodies indicated, followed by immunoblotting with the antibodies indicated or kinase assay for PI. Labeled PI (*PIP*) was subjected to thin layer chromatography and autoradiography as described previously (20) (*G*). Each *bar* represents the mean ± S.E. (n = 5–10). *, $p < 0.05$; **, $p < 0.01$ compared with untreated wild-type mice.

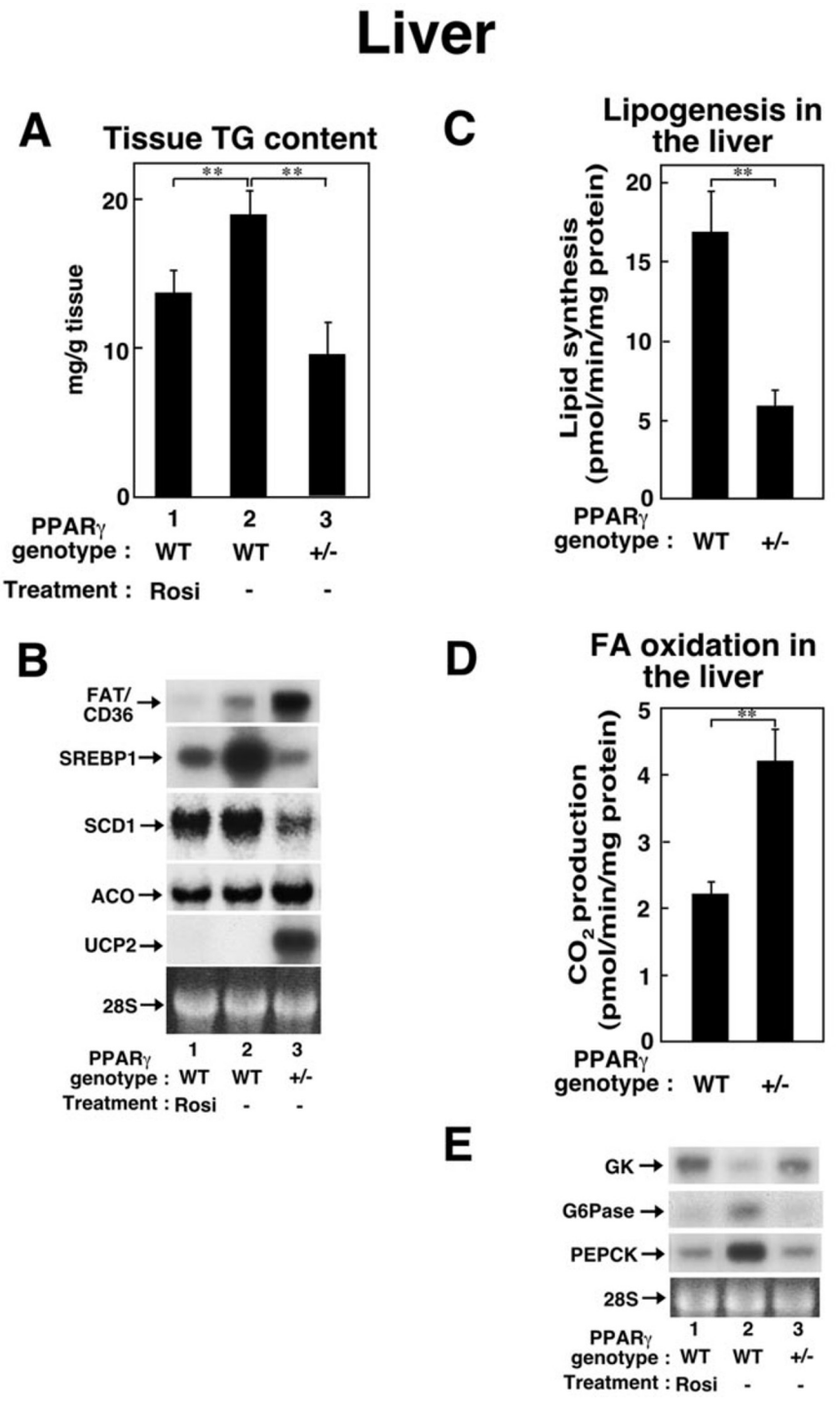

FIG. 5. **TZD indirectly decreases molecules involved in FFA influx into the liver, whereas heterozygous PPARγ deficiency combusts fatty acid and decreases lipogenesis, thereby both decreasing tissue TG content in liver.** Tissue triglyceride (*A*), amounts of the mRNAs of fatty-acid translocase (*FAT*)/CD36, SREBP 1, stearoyl Co-A desaturase (*SCD*) 1, acyl-CoA oxidase (*ACO*), and uncoupling protein (*UCP*) 2 (*B*), lipid synthesis from [^{14}C]acetate in the liver (*C*), fatty acid (*FA*) oxidation (*D*), glucokinase, phosphoenolpyruvate carboxykinase (*PEPCK*) and glucose-6-phosphatase (*G6Pase*) (*E*), in liver of wild-type (*WT*) and heterozygous PPARγ-deficient mice (+/−) untreated (−) or treated with rosiglitazone (*Rosi*) for 4 weeks while on the HF diet. Rosiglitazone was given as a 0.01% food admixture. Fatty acid oxidation was assessed by the measurements of [^{14}C]CO_2 production from [^{14}C]palmitic acid (*D*). Each bar represents the mean ± S.E. (n = 5–10). *, $p <$ 0.05; **, $p < 0.01$; compared with untreated wild-type mice.

heterozygous PPARγ deficiency, both of which resulted in protection against HF diet-induced adipocyte hypertrophy, significantly decreased FFA and TNFα (Fig. 3, *G* and *H*) and increased adiponectin (Fig. 3*I*) and at the same time ameliorated insulin resistance (Fig. 1, *D* and *E*) on the HF diet. However, treatment of wild-type mice with a PPARγ agonist increased adipose tissue mass (Fig. 1*C*, *lane 1*) and body weight (Fig. 1*B*, *lane 1*), whereas heterozygous PPARγ deficiency significantly decreased them (Fig. 1, *B* and *C*, *lane 3*). These findings raised the possibility that levels of expression of molecules regulating insulin sensitivity may be more closely related to adipocyte size than PPARγ activity, adipose tissue mass, or body weight *in vivo*. Large adipocytes are known to be resistant to anti-lipolytic action of insulin, thereby releasing a large amount of FFA (30); however, the mechanisms underlying the correlation between larger adipocytes and up-regulation of TNFα and/or down-regulation of adiponectin remain to be elucidated.

TZD Indirectly Decrease Molecules Involved in Fatty Acid Influx into Muscle/Liver, whereas Heterozygous PPARγ Deficiency Increases Fatty Acid Combustion and Decreases Lipogenesis via Increased Leptin Effect, Thereby Both Decreasing Their Tissue TG Content—Interestingly, both supraphysiological activation of PPARγ and moderate reduction of PPARγ activity significantly reduced tissue TG content in muscle and liver (Fig. 4*A* and Fig. 5*A*), suggesting that insulin resistance has an excellent correlation with tissue TG content in muscle and liver (Fig. 1, *D* and *E*, Fig. 4*A,* and Fig. 5*A*) (15).

In wild-type mice treated with rosiglitazone, the decreased

tissue TG content in muscle/liver, where PPARγ was less abundantly expressed as compared with what was in WAT, was presumably via reduced expression of molecules involved in FFA influx into muscle/liver (Fig. 4*E* and Fig. 5*B, lanes 1* and *2*). On the other hand, heterozygous PPARγ deficiency reduced expression of lipogenic enzymes such as SCD1 (Fig. 4*E* and Fig. *5B, lanes 2* and *3*) and SREBP 1 (Fig. 5*B*, *lanes 2* and *3*), and indeed significantly reduced lipogenesis (Fig. 5*C*), presumably due to increased leptin effects (Fig. 2, *B–D*) (28), may reduce tissue TG content in muscle/liver (Fig. 4*A* and Fig. 5*A, lanes 2* and *3*).

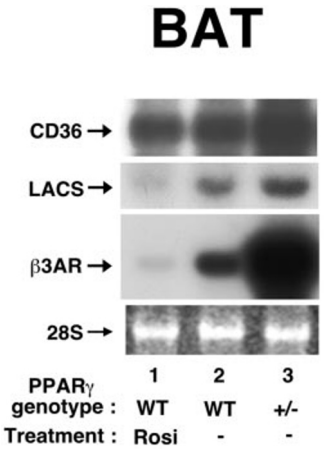

FIG. 6. **Heterozygous PPARγ deficiency increases expression of molecules involved in β-oxidation and β_3-adrenergic receptor in BAT.** Amounts of the mRNAs of fatty-acid translocase (*FAT*)/CD36, long chain acyl-CoA synthetase (*LACS*), and β_3-adrenergic receptor (*AR*) in BAT of wild-type (*WT*) and heterozygous PPARγ-deficient mice (+/−) untreated (−) or treated with rosiglitazone (*Rosi*) for 4 weeks while on the high fat diet. Rosiglitazone was given as a 0.01% food admixture.

Heterozygous PPARγ Deficiency Increases Fatty Acid Combustion and Molecules Involved in Energy Dissipation via PPARα Pathways in Liver, Muscle, and BAT—Moreover, in muscle/liver from heterozygous PPARγ-deficient mice, increased expression of enzymes involved in β-oxidation such as acyl-CoA oxidase and that of molecules involved in energy dissipation such as UCP2 (Fig. 4*E* and Fig. 5*B*, *lanes 2* and *3*) were observed. Fatty acid oxidation was indeed significantly increased in muscle/liver from heterozygous PPARγ-deficient mice as compared with that in wild-type mice on the HF diet (Fig. 4*F* and Fig. 5*D*). These alterations may be an additional mechanism for reduced TG content in muscle/liver of heterozygous PPARγ-deficient mice. Since these effects were recapitulated by treatment of wild-type mice with Wy-14,643, a PPARα agonist as reported (18, 19, 31) (data not shown), PPARα pathways appeared to be activated in the liver of heterozygous PPARγ-deficient mice.

In the BAT, where PPARα was relatively abundantly expressed compared to that in WAT, significant increases in the expression of molecules involved in fatty acid combustion presumably via activation of PPARα pathways (18, 19, 31) and β_3-adrenergic receptor (Fig. 6), due to increased leptin effects (28) and decreased PPARγ effects (29), were observed. These alterations in concert may provide the mechanism that increased energy expenditure by heterozygous PPARγ deficiency (Fig. 2*A*).

Heterozygous PPARγ Deficiency Indeed Improves Insulin Signal Transduction and Insulin Actions in Each Target Organ—Increased tissue TG content has been reported to interfere with insulin-stimulated phosphatidylinositol (PI) 3-kinase activation and subsequent GLUT4 translocation and glucose uptake (15). Next, we tried to experimentally clarify the relationships between tissue TG content and insulin resistance. To do so, we increased tissue TG content by high fat feeding, leptin

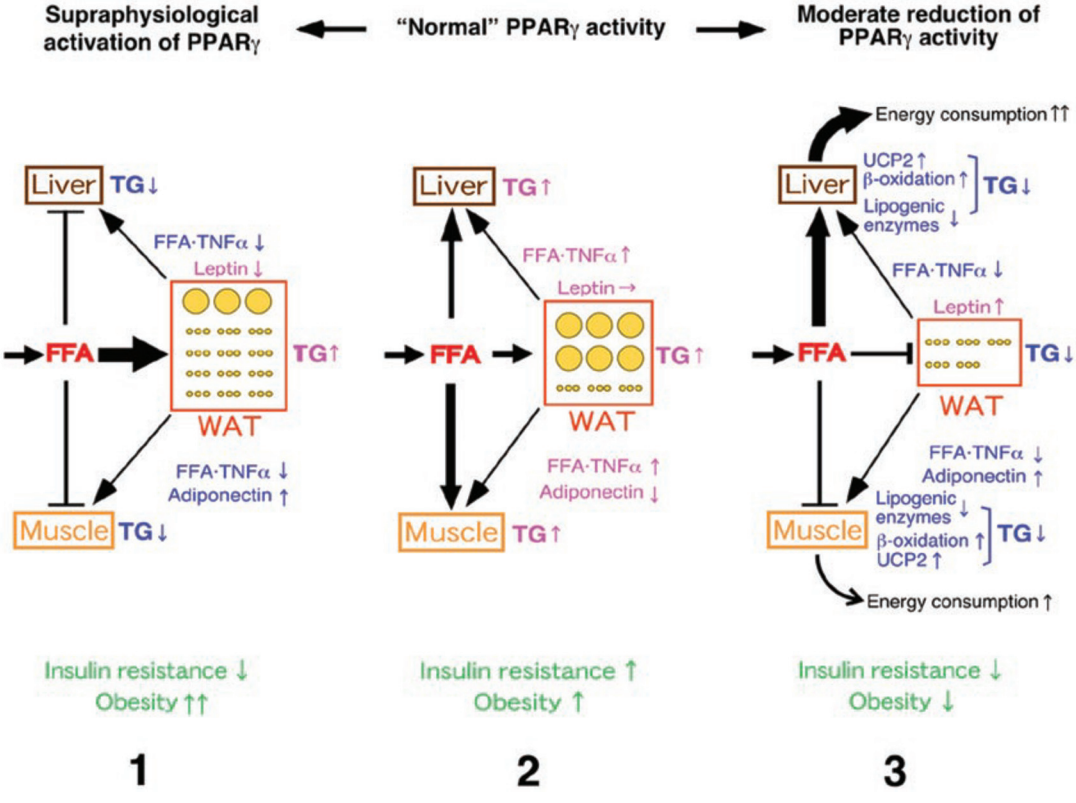

FIG. 7. **Proposed mechanisms for the regulation of insulin sensitivity and adiposity by PPARγ.** See "Both Heterozygous PPARγ Deficiency and PPARγ Agonist Improve Insulin Resistance Presumably Due to Decreased TG Content in Muscle/Liver as Well as Prevention of Adipocyte Hypertrophy" under "Discussion."

receptor deficiency, or agouti overexpression. The tissue TG content of skeletal muscle and insulin resistance were increased in mice on the HF diet compared with those in mice on the HC diet (Fig. 4*B*). The tissue TG content of skeletal muscle and insulin resistance of db/db mice were also increased compared with their wild-type controls on both the HC and HF diets (Fig. 4*B*). We obtained essentially similar results by using KKAy mice and their wild-type controls (KK) (Fig. 4*C*). These findings raise the possibility that increases in tissue TG content are associated with insulin resistance. Conversely, decreased tissue TG content due to decreased lipid synthesis and increased fatty acid oxidation in muscle/liver from heterozygous PPARγ-deficient mice (Fig. 4*F* and Fig. 5, *C* and *D*) may cause an increase in insulin sensitivity (Fig. 4*D*). Shulman and co-workers (32) proposed a cause and effect relationship between the accumulation of intracellular fatty acid-derived metabolites and insulin resistance. However, there are instances in which tissue TG content actually does not change in another scenario that also causes insulin resistance, *i.e.* adipose-selective targeting of the GLUT4 gene (32). Thus, interpretation should be done with caution, and decreased tissue TG content in muscle/liver is one possible mechanism for the results of increased insulin sensitivity in heterozygous PPARγ-deficient mice.

Consistent with this possibility, decreased TG content in muscle of heterozygous PPARγ-deficient mice indeed improved insulin signal transduction in muscle, as demonstrated by increases in insulin-induced tyrosine phosphorylation of insulin receptor, insulin receptor substrate (IRS)-1 and IRS-2, and insulin-stimulated PI3-kinase activity in phosphotyrosine, IRS-1 and IRS-2 immunoprecipitates, and insulin-stimulated Akt activity in skeletal muscle (Fig. 4*G*). The reduction of TG content in liver of heterozygous PPARγ-deficient mice was associated with increased expression of glucokinase and decreased expression of enzymes involved in gluconeogenesis such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (Fig. 5*E*), indicating increased insulin actions also in liver.

DISCUSSION

Both Heterozygous PPARγ Deficiency and PPARγ Agonist Improve Insulin Resistance Presumably Due to Decreased TG Content in Muscle/Liver as Well as Prevention of Adipocyte Hypertrophy—We attempted to explain how insulin resistance could be improved by the following two opposite PPARγ activity states: a potent activation of PPARγ and its moderate reduction. We did so by using heterozygous PPARγ-deficient mice and a pharmacological activator of PPARγ in wild-type mice. On the basis of experimental results obtained in this study, we propose the following hypothesis on the mechanisms for the regulation of insulin sensitivity by PPARγ (Fig. 7).

As shown in the Fig. 7, *panel 2*, on the HF diet, "normal" amounts of PPARγ activity seen in wild-type mice increase TG content in WAT, skeletal muscle, and liver due to a combination of increased fatty acid influx into WAT, skeletal muscle, and liver and HF diet-induced leptin resistance, leading to insulin resistance and obesity. Moreover, hypertrophic adipocytes may increase the secretion of molecules causing insulin resistance, such as FFA (15) and TNFα (16), and decrease that of an insulin-sensitizing hormone, such as adiponectin (17).

As shown in Fig. 7, *panel 1*, supraphysiological activation of PPARγ way beyond that by TZD stimulates adipogenesis, which promotes a flux of FFA from liver and muscle into WAT, leading to a decrease in TG content in liver and muscle and improvement of insulin sensitivity at the expense of increased WAT mass, *i.e.* obesity. Moreover, TZD induce adipocyte differentiation and apoptosis, thereby increasing the number of small adipocytes, which finally lead to alleviation of insulin resistance presumably via a decrease in molecules causing insulin resistance, such as FFA and TNFα, and up-regulation of insulin-sensitizing hormone adiponectin, at least in part.

By contrast, as shown in the Fig. 7, *panel 3*, moderate reduction of PPARγ activity observed in untreated heterozygous PPARγ-deficient mice decreases TG content in WAT, skeletal muscle, and liver. This effect is due to a combination of increased leptin expression by antagonism of PPARγ-mediated suppression of the gene, thereby reducing expression of lipogenic enzymes, and consequent activation of PPARα pathway in liver, BAT, and skeletal muscle, leading to an increase in expression of UCP2 and enzymes involved in β-oxidation. These observations fit well with the recently demonstrated effects of PPARα agonists on insulin resistance (33) and decreased fatty acid combustion in PPARα-deficient mice (34). Moreover, direct antagonism of PPARγ to reduce lipogenesis in WAT prevents adipocyte hypertrophy under the HF diet, thereby reducing the molecules causing insulin resistance, such as FFA and TNFα, and up-regulating the insulin-sensitizing hormone adiponectin, at least in part. These alterations lead to prevention against HF diet-induced obesity and insulin resistance. The data showing that moderate reduction of PPARγ activity resulted in increased insulin sensitivity were further confirmed by the observation that treatment of heterozygous PPARγ-deficient mice with a low dose of TZD caused the re-emergence of insulin resistance (9).

This study has thus revealed the mechanisms whereby both PPARγ agonist and heterozygous PPARγ deficiency have a similar effect on insulin sensitivity. However, it should also be noted that PPARγ agonist and heterozygous PPARγ deficiency have an opposite effect on adiposity and energy expenditure which appear to be more directly regulated by PPARγ activity.

Both Heterozygous PPARγ Deficiency and TZD Prevent Adipocyte Hypertrophy via Different Mechanisms, Thereby Finally Contributing to Increased Insulin Sensitivity—Although both heterozygous PPARγ deficiency and PPARγ agonist finally improve insulin resistance via decreased TG content in muscle/liver and prevention of adipocyte hypertrophy, there are some important differences between them. First, although both reduced TG content in muscle/liver, heterozygous PPARγ deficiency did so via activation of fatty acid combustion and energy dissipation, whereas TZD did so via potent stimulation of adipogenesis, thereby increasing fatty acid flux from muscle/liver into WAT. Second, both prevented HF diet-induced adipocyte hypertrophy, and TZD markedly increased the number of newly differentiated small adipocytes, whereas heterozygous PPARγ deficiency appeared not to change the total number of adipocytes.

Taken together, all of these differences are consistent with the notion that activation of PPARγ plays a role in energy storage and adiposity, and reduction of PPARγ causes energy dissipation and prevention of adiposity.

In conclusion, although by different mechanisms, both heterozygous PPARγ deficiency and PPARγ agonist improve insulin resistance via decreased TG content in muscle/liver and prevention of adipocyte hypertrophy (Fig. 7).

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