PPARδ regulates glucose metabolism and insulin sensitivity

Chih-Hao Lee*, Peter Olson†, Andrea Hevener‡, Isaac Mehli, Ling-Wa Chong‡, Jerrold M. Olefsky§, Frank J. Gonzalez†, Jungyeob Ham*, Heonjoong Kang*, Jeffrey M. Peters‡, and Ronald M. Evans†,‡,‡‡

*Department of Genetics and Complex Diseases, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115; †Howard Hughes Medical Institute, Gene Expression Laboratory, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037; Departments of ‡Biology and §Medicine, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92039; ‡Laboratory of Metabolism, Division of Basic Sciences, National Cancer Institute, Bethesda, MD 20892; **Marine Biotechnology Laboratory, School of Earth and Environmental Sciences and Center for Marine Natural Products Drug Discovery, Seoul National University, Seoul 151-747, Korea; and ‡‡Department of Veterinary Science and Center for Molecular Toxicology and Carcinogenesis, Pennsylvania State University, University Park, PA 16802

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The metabolic syndrome is a collection of obesity-related disorders. The peroxisome proliferator-activated receptors (PPARs) regulate transcription in response to fatty acids and, as such, are potential therapeutic targets for these diseases. We show that PPARδ (NR1C2) knockout mice are metabolically less active and glucose-intolerant, whereas receptor activation in db/db mice improves insulin sensitivity. Euglycemic–hyperinsulinemic-clamp experiments further demonstrate that a PPARδ-specific agonist suppresses hepatic glucose output, increases glucose disposal, and inhibits free fatty acid release from adipocytes. Unexpectedly, gene array and functional analyses suggest that PPARδ ameliorates hyperglycemia by increasing glucose flux through the pentose phosphate pathway and enhancing fatty acid synthesis. Coupling increased hepatic carbohydrate catabolism with its ability to promote β-oxidation in muscle allows PPARδ to regulate metabolic homeostasis and enhance insulin action by complementary effects in distinct tissues. The combined hepatic and peripheral actions of PPARδ suggest new therapeutic approaches to treat type II diabetes.

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yndrome X, or metabolic syndrome, includes several disorders resulting from metabolic dysregulation (1). Among these, insulin resistance alone affects millions of people worldwide. This disease is characterized by a compromised ability of insulin to control glucose and lipid metabolism, which results in decreased glucose disposal to muscle, increased hepatic glucose production, and overt postprandial hyperglycemia. When left untreated, insulin resistance leads to the development of type II diabetes and related complications, including cardiovascular disease, which are leading causes of death.

The PPARs are members of the nuclear receptor superfamily, which are ligand-modulated transcription factors that regulate gene expression programs of many important biological processes (2). The three PPARs, α (NR1C1), β/δ (NR1C2), and γ (NR1C3), are activated by ligands and are targets for current and prospective drug therapies for components of the metabolic syndrome (3, 4). PPARα, a target for the fibrate class of lipid-lowering drugs (5–7), is primarily expressed in liver, where it up-regulates genes involved in lipid oxidation in the fasted state (8, 9). PPARγ is highly expressed in adipose tissue and regulates adipogenesis and insulin sensitivity (10–14). Thiazolidinediones are a class of drugs that increase insulin sensitivity through activating PPARγ (15). PPARδ is expressed in many tissues, including metabolically active sites such as liver, muscle, and fat, and its role in the metabolic syndrome is only now being elucidated (16, 17). Treatment with a high-affinity PPARδ agonist GW501516 has been shown to increase high-density lipoprotein cholesterol (18, 19), affect lesion progression in a mouse model of atherosclerosis (20), cause weight loss, and regulate muscle fiber type-switching when constitutively activated (21, 22). In the latter two cases, the phenotypes appeared to be mediated through up-regulation of lipid catabolism and oxidative phosphorylation in fat and muscle. In addition, ligands for PPARδ have been proposed to be potential insulin sensitizers, based on improvements in standard glucose-tolerance tests (21, 23). These studies, however, used long-term ligand-treatment regimens that resulted in significant weight loss and a decrease in fat mass. These effects alone enhance insulin sensitivity. Therefore, it remains unclear whether PPARδ can directly regulate insulin sensitivity and, if so, through which tissue and what mechanism.

In this study, we sought to determine the effect of a PPARδ synthetic ligand in animals that have already developed insulin resistance but under conditions in which body weight, food intake, and serum lipid profiles are minimally affected after drug intervention. We show that PPARδ knockout mice are glucose intolerant, whereas treatment of diabetic db/db mice with a high-affinity PPARδ agonist improves insulin sensitivity in all major insulin-responsive tissues. Molecular and functional analyses suggest that PPARδ activation reduces hepatic glucose output by increasing glycolysis and the pentose phosphate shunt and promoting fatty acid synthesis in the liver. This uncovered hepatic activity thus constitutes the earliest component of the regulatory mechanism by which PPARδ regulates insulin sensitivity, in addition to its known function in fatty acid β-oxidation.

Results

PPARδ Mutant Mice Are Metabolically Less Active and Glucose Intolerant. To explore PPARδ’s role in the onset of the metabolic syndrome, we examined a cohort of 6-month-old male PPARδ null mice along with age-matched wild-type controls in metabolic cages. Although PPARδ−/− mice have been shown to gain less weight and exhibit defects in hepatic TG production at a younger age (17, 24), the weight difference normalized in older cohorts, and no significant changes were detected in levels of circulating Acrp30, insulin, glucose, and lipids between the control and mutant group (see Table 3, which is published as supporting information on the PNAS web site). In addition, these weight-normalized null mice consumed less food, water, and oxygen and produced less carbon dioxide than control animals when fed a standard chow diet (Fig. 1A and B). Metabolic rate (presented as heat) was also decreased, indicating that these animals expended less energy (Fig. 1C). The respiratory exchange ratio [(RER) = VCO2/VO2], which indicates

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Abbreviations: GW, GW501516; Q-PCR, quantitative PCR; RER, respiratory exchange ratio; TG, triglyceride.

1C.-H.L. and P.O. contributed equally to this work.

1To whom correspondence should be addressed. E-mail: evans@salk.edu.

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whether lipids (RER = 0.7) or carbohydrates (RER = 1) are being oxidized, was then determined. Wild-type animals went from burning \( \approx 70\% \) of their fuel from lipids during the fasted state to nearly 100% from carbohydrates in the fed state (Fig. 1D; and see Fig. 5A, which is published as supporting information on the PNAS web site, for statistical analysis). Null mice, on the other hand, had significantly lower RER values, particularly during the fed state, suggesting a defect in switching their energy source. This observation prompted us to investigate glucose tolerance in the null animals, because one of the early signs of diabetes is a blunted response to insulin-stimulated glucose utilization in the fed state. After a 6-hour fast, both genotypes had similar baseline glucose concentrations (Fig. 1F). When subjected to a glucose-tolerance test, which evaluates the ability of the body to adjust glucose levels after an acute glucose injection, glucose levels of wild-type mice peaked at 20 min and rapidly returned to the basal level. In contrast, null animals showed a delayed response, indicating glucose intolerance. Both wild-type and null animals (n = 8) were then fed a high-fat, high-carbohydrate (HF) diet for 10 weeks to induce insulin resistance, followed by treatment with either GW501516 (GW), a high-affinity PPAR\( ^{\gamma} \) agonist, at 4 mg/kg/day or DMSO alone (four mice from each genotype) for 2 weeks. Both groups gained similar weight on this diet. Ligand treatment for 2 weeks also did not affect weight gain in either genotype (see Fig. 6A, which is published as supporting information on the PNAS web site). Because long-term treatment with this compound leads to weight loss, which affects the dynamics of metabolism (21, 23), this experimental design allowed us to directly study the acute effect of PPAR\( ^{\gamma} \) activation on glucose tolerance.

In agreement with previous reports, RER values remained low (25) \((=0.7)\) throughout the 24 h (Fig. 6B) because of the high fat content (35% by weight) in the diet. To test the efficacy of GW to improve glucose tolerance and whether the effect is receptor-dependent, all four groups were then subjected to a glucose-tolerance test (GTT). Both wild-type and null mice displayed hyperglycemia (220.6 \(\pm\) 14.0 in wild-type vs. 215.9 \(\pm\) 14.2 in null mice, \(P = 0.8)\). Interestingly, both groups were similarly glucose-intolerant after high-fat, high-carbohydrate diet treatment (Fig. 1F). Wild-type mice treated with GW, however, had a lower fasting glucose level (225.0 \(\pm\) 11.4 in DMSO vs. 182.5 \(\pm\) 2.9 in GW, \(P = 0.03)\), and their response to the glucose challenge was greatly improved. Importantly, this improvement in the GTT by GW treatment was completely abolished in the null mice, demonstrating that this effect is receptor-dependent. These data support the proposal that PPAR\( ^{\gamma} \) functions as a mediator of insulin sensitivity.

### Table 1. Metabolic parameters of db/db mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle</th>
<th>GW501516</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain, g</td>
<td>5.91 (\pm) 0.56</td>
<td>6.06 (\pm) 0.46</td>
</tr>
<tr>
<td>Liver/body weight</td>
<td>0.058 (\pm) 0.001</td>
<td>0.072 (\pm) 0.003**</td>
</tr>
<tr>
<td>Fat/body weight</td>
<td>0.039 (\pm) 0.001</td>
<td>0.039 (\pm) 0.002</td>
</tr>
<tr>
<td>Serum lipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>300.42 (\pm) 37.58</td>
<td>272.61 (\pm) 34.17</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>123.58 (\pm) 6.69</td>
<td>164.23 (\pm) 9.69**</td>
</tr>
<tr>
<td>HDL-c, mg/dl</td>
<td>68.20 (\pm) 3.97</td>
<td>94.21 (\pm) 6.05**</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>594.4 (\pm) 45.6</td>
<td>396.0 (\pm) 41.4*</td>
</tr>
<tr>
<td>Fasting insulin, ng/ml</td>
<td>298.0 (\pm) 27.1</td>
<td>229.3 (\pm) 17.0*</td>
</tr>
<tr>
<td>Acyrp30, mg/ml</td>
<td>7.20 (\pm) 0.73</td>
<td>9.00 (\pm) 1.08</td>
</tr>
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</table>

Mice were 10 weeks old at the start of the 2-week drug treatment. HDL-c, high-density lipoprotein cholesterol.

1) 8-week-old mice at the start of the 2-week drug treatment. *, \(P < 0.05\); **, \(P < 0.005\).

PPAR\( ^{\gamma} \) Activation Increases Hepatic and Peripheral Insulin Sensitivity. To determine whether PPAR\( ^{\gamma} \) acts as an insulin sensitizer, we next examined the activity of GW on db/db mice, a well-established model to study diabetes. These animals become insulin resistant on normal chow, which also allowed us to examine RER. Ten 10-week-old male db/db mice were treated with either 4 mg/kg/day GW or DMSO for two weeks. This regimen did not cause changes in weight gain, food intake, or levels of circulating free fatty acids, whereas total and high-density lipoprotein cholesterol was increased with GW treatment (Table 1). In line with the decreased TG level observed after long-term treatment (21, 23), serum TG levels were moderately lower in the GW-treated group (Table 1 and data not shown). However, the difference was not statistically significant. The liver-to-body-weight ratio was increased in the GW-treated group, described in ref. 23. GW treatment significantly increased RER, particularly during the fed state, indicating increased carbohydrate usage. Consistent with results from the HF study, GW treatment dramatically lowered fasting glucose and enhanced glucose tolerance (Fig. 2B and Table 1). Similarly, GW also increased the ability of insulin to lower glucose in the insulin-tolerance test (Fig. 2C), demonstrating improved insulin sensitivity. Although there was a decrease in fasting serum

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insulin (27.2 vs. 20.9 ng/ml) in the GW-treated group, it was not statistically significant. However, ligand treatment decreased insulin levels in a younger cohort in which insulin values were less variable within the groups (see below).

To further define which tissues have improved insulin sensitivity and to what extent, we performed euglycemic–hyperinsulinemic clamp. The animals used for this study were 8 weeks old. After a 2-week treatment, GW had no effect on body weight (35.9 vs. 34.6 g; P = 0.13 g vs. 34.6 + 0.2 g; P = 0.613) but significantly lowered fasting blood glucose and insulin levels (23.0 vs. 7.4 ng/ml) (Table 1). As predicted from these reductions, basal glucose turnover was decreased by 35% (P = 0.028, Fig. 2E, vehicle vs. GW at the basal state), and the exogenous glucose infusion rate (GIR) required to maintain euglycemia was markedly elevated by 57% (P = 0.04) in the GW-treated mice (Fig. 2D). A good portion of the difference in GIR could be accounted for by the insulin-stimulated reduction in hepatic glucose production (P = 0.016; Fig. 2E, vehicle vs. GW in the clamped state), which was improved by 47% and represents improved hepatic insulin sensitivity. Insulin-stimulated glucose disposal was increased ~2-fold by the drug (Fig. 2F), demonstrating that this compound also improved peripheral insulin sensitivity. In addition, GW treatment also led to enhanced insulin response in adipocytes, as demonstrated by a 33% reduction in serum free fatty acids after the clamp (Fig. 2G).

**PPARα Regulates Critical Transcriptional Programs of Glucose and Fatty Acid Metabolism in Liver.** To determine the molecular mechanism underlying the insulin-sensitizing activity of PPARα activation, we conducted DNA array analysis to compare gene expression profiles of liver, adipocytes, and muscle between control and GW-treated db/db mice. The results obtained were confirmed by either quantitative-PCR (Q-PCR) or Northern blot (Table 2; and see Fig. 7, which is published as supporting information on the PNAS web site). Several key genes were also similarly regulated in samples from high-fat-diet-induced insulin-resistant mice, further confirming the importance of these target genes (Fig. 7B). Unexpectedly, there were no significant differences in the expression of relevant genes in adipocytes, whereas carnitine palmitoyltransferase 1 (CPT1), a key gene in fatty acid β-oxidation, was identified as a target in muscle as described in refs. 21 and 23. The most responsive tissue was the liver, where clusters of genes involved in fatty acid synthesis were up-regulated by ligand treatment (Table 2). These genes can be further divided into three groups. The first group is comprised of genes that are directly responsible for fatty acid synthesis, including acetyl-CoA carboxylase β (ACCβ), fatty acid synthase (FAS), acyl-CoA thioesterase 1, and ATP citrate lyase. The second group consists of genes involved in elongation and modification of fatty acids including ELOVL family member 6 (ELOVL6), stearoyl-CoA desaturase 2 (SCD2), and glycerol-3-phosphate acyltransferase (GPAT). The third group includes genes such as malic enzyme in the pyruvate/malate cycle and phosphogluconate dehydrogenase (PGD) in the pentose phosphate pathway that mediates NADPH generation to provide reducing power for lipid synthesis. It is interesting to note that both fatty acid synthesis and the pentose phosphate pathway use glucose or its metabolites as substrates. It has been demonstrated that overexpression of glucokinase in liver increases glycolysis by activating the pentose phosphate shunt with concomitant up-regulation of lipogenic genes, including ACC1 and FAS, resulting in reduced hepatic glucose production, increased fatty acid oxidation in muscle, and improved peripheral insulin sensitivity (26). This suggests that PPARα may increase glucose catabolism through these processes.

Other genes that are involved in lipid transport (CD36 and phospholipid transfer protein, PLTP) and cholesterol synthesis (mevalonate decarboxylase) were also identified from the array. However, it appeared that cholesterol synthesis was not altered, because the expression of the rate-limiting enzyme HMG-CoA reductase remained unchanged (Fig. 3A), and there was no difference in liver cholesterol content (Fig. 4C). GW treatment may also inhibit glycolysis through suppressing protein phosphatase 1 regulatory subunit (PPP1R1C) expression. We also examined the expression of phosphoenolpyruvate carboxykinase (PEPCK), a rate-limiting enzyme for gluconeogenesis, which was not included in the array collection, and found a moderate reduction in GW-treated samples (Fig. 7A). Interestingly, we did not detect expression changes in known PPARα targets, such as acetyl-CoA oxidase and CPT1 in liver (Figs. 4 and 7A), indicating that these two receptors regulate distinct transcriptional programs.

Recently, it has been shown that liver X receptors (LXRα and -β) regulate lipogenesis through transcriptional induction of sterol regulatory element-binding protein (SREBP)-1c (27, 28), a master regulator of hepatic lipid metabolism. To rule out the possibility that the observed regulation was indirectly mediated by SREBP-1c, we analyzed its expression in liver by Q-PCR and found no changes between control and GW-treated groups (Fig. 3A). We next examined the activity of the ACCβ promoter in a reporter transient transfection assay. The human ACCβ gene is controlled by two promoters (29) (Fig. 3B). SREBP-1c is known to induce its expression through promoter II. However, PPARα did not affect the activity of a reporter driven by this promoter [PII (1.3 kb)] when cotransfected into HepG2 cells (Fig. 3C). In contrast, we found that promoter I [PI (0.58 kb)] could be activated by PPARα. Subsequently, a putative PPAR response element was identified at −480 to −468 (relative to the transcriptional start site). Mutation [PI (0.58 kb/M)] or deletion of this element completely abolished the ability of PPARα to
induce the promoter activity (Fig. 3C and data not shown). These results suggest that ACCβ is a target gene of PPARδ and indicate that PPARδ controls hepatic fatty acid synthesis through direct transcriptional control.

**PPARδ Increases Glucose Consumption, Activates the Pentose Phosphate Shunt, and Promotes Fatty Acid Synthesis.** Previous studies and our array data have demonstrated a critical role of PPARδ in peripheral fatty acid oxidation (21, 23). However, this beneficial activity alone does not explain how PPARδ improves insulin sensitivity. Therefore, the regulation of the glycolytic pathways mediated by the pentose phosphate shunt and lipogenesis appears to constitute the second component of the insulin-sensitizing action of PPARδ. To test this hypothesis, we performed a series of in vitro functional assays. First, we measured the activity of hepatic glucose-6-phosphate dehydrogenase (G-6-PDH), the rate-limiting enzyme in the pentose phosphate pathway, and found a 2-fold (100%) increase in livers of GW-treated db/db mice compared with the control group (Fig. 4A). The flux from glucose to fatty acid synthesis was then directly determined in primary hepatocytes isolated from both vehicle- and GW-treated db/db mice by [14C]glucose labeling. Indeed, there was also an ~2-fold increase in the conversion rate of 14C-labeled glucose into organically extractable lipids (TGs/fatty acids) in GW-treated cells (Fig. 4B). Consistent with the functional analyses, we detected a 20% increase in liver TG content in GW-treated animals, whereas cholesterol and glycogen content was not affected (Fig. 4C and data not shown).

Lastly, we examined fatty acid catabolism in isolated soleus muscle strips and found GW treatment significantly increased the $\beta$-oxidation rate (Fig. 4D), which is consistent with results from previous reports (21, 23) and the up-regulation of CPT-1 in muscle. In summary, these data reveal a previously unrecognized role of PPARδ in the liver and suggest that PPARδ regulates metabolic homeostasis and insulin sensitivity through a unique mechanism, which consists of a fat-production component in liver to consume glucose and a counterbalancing fat-burning component in muscle to reduce lipid burden.

**Discussion**

This study utilizes metabolic, genetic, and pharmacologic approaches to examine the role of PPARδ in regulating insulin sensitivity. We identify liver as a major PPARδ-responsive tissue and reveal an unexpected glucose-burning pathway, which contributes to the ability of PPARδ agonists to alleviate hyperglycemia and improve insulin sensitivity.

Based on the glucose–fatty acid cycle proposed by Randle (30), an induction in fatty acid oxidation should result in decreased carbohydrate usage. This hypothesis raises an intriguing question as to how activation of PPARδ, a fatty acid receptor known to activate lipid oxidation, enhances glucose utilization. The answer seems to reside in the ability of PPARδ to promote glucose flux through the pentose phosphate shunt and stimulate fatty acid synthesis in liver. The former generates reducing power (NADPH) needed for lipid synthesis and can consume up to 20% of hepatic glucose (31, 32). The importance of the pentose phosphate pathway and lipogenesis in regulating hepatic glucose output and systemic metabolic homeostasis has emerged in recent studies. It has been demonstrated that deletion of the GLUT4 gene leads to activation of hepatic fatty acid synthesis/pentose phosphate pathway and increased skeletal-muscle $\beta$-oxidation, an energy substrate-switching phenotype, because of the inability of the body to use glucose. Thus, fats were made at the expense of glucose in the liver to be consumed in muscle, providing a mechanism how GLUT4 knockout mice maintain normal glucose levels and insulin sensitivity (33). Importantly, adenosine-mediated glucokinase overexpression drives glycolysis through the fatty acid synthesis/pentose phosphate pathway, resulting in reduced hepatic glucose production, increased $\beta$-oxidation in muscle, and improved systemic insulin sensitivity (26). It appears that PPARδ activation exerts an “energy substrate-switching” phenotype, suggesting that this receptor is
capable of controlling the substrate utilization through regulating distinct transcriptional programs in different tissues, which represents a unique mechanism for metabolic regulation. The increase in TG raises the concern as to whether long-term drug treatment will cause hepatic steatosis. However, we did not observe signs of fatty liver with treatment up to 6 months in wild-type C57BL/6 mice (data not shown). It is likely that the increased β-oxidation in muscle results in a net decrease in the fat content. Indeed, long-term treatment has been shown to reduce body weight and levels of circulating and liver TG content. Indeed, long-term treatment has been shown to increase body weight and levels of circulating and liver TG content. Consistent with this idea, pharmacological activation of PPARγ and PPARδ increases insulin sensitivity through regulating different metabolic pathways. Unlike thiazolidinedione treatment, which increases body weight, reduces serum fatty acid, and increases levels of Acrp30 (3), PPARδ agonists drastically improve insulin sensitivity without affecting these parameters (Table 1). In addition, Q-PCR analyses demonstrated that the expression of PPARγ target genes in adipocytes, such as TNFα, GLUT4, and CD36 was unaffected by GW treatment (see Fig. 8, which is published as supporting information on the PNAS web site). Our data suggests that activation of PPARδ promotes a metabolic shift to reduce hepatic glucose output, enabling an enhancement of insulin sensitivity. It is likely, however, that PPARδ would exert a more profound effect on muscle and adipocyte with prolonged ligand treatment as proposed in refs. 21 and 22. Together with the PPARδ loss-of-function data, our study implicates PPARδ as a bona fide target to treat insulin resistance and as a candidate for population-based screening for genetic polymorphisms that predispose to this disease.

**Materials and Methods**

**Animal Experiments.** PPARδ knockout mice were generated in two laboratories in different genetic backgrounds (16, 17). PPARδ null animals in the C57BL/6 background were used for metabolic studies (eight age-matched 6-month-old male PPARδ null and wild-type mice) (17). These mice were then challenged with a high-fat, high-carbohydrate diet (F3282, Bio-Serv, Frenchtown, NJ) for 10 weeks to induce insulin resistance. Four mice from each group were given either vehicle or ligand for an additional 2 weeks. GW501516 compound was dissolved in DMSO and suspended in 0.5% carboxymethylcellulose. Mice were gavaged at the dose of 4 mg/kg/day based on a previous report (18). Db/db mice were purchased from The Jackson Laboratory. Ten db/db male mice (10 weeks old) were used for both control and ligand-treated groups for metabolic studies, whereas six mice (8 weeks old) for each group were included in the clamp study. These mice were also given the ligand at 4 mg/kg/day for 2 weeks.
Metabolic Studies. Metabolic cage studies were conducted in a Comprehensive Lab Animal Monitoring System (12-chamber CLAMS system, Columbus Instruments, Columbus, Ohio). Serum and plasma were collected after 6-h fasting. Total cholesterol, TG, and free fatty acids were determined by using enzymatic reactions (Thermo Fisher, Waltham, MA; free fatty acid, Wako Chemicals, Richmond, VA). The activity of G-6-PDH in liver lysates from vehicle or GW-treated mice was determined by using NADPH produced (OD490) within a period of 5 min by using a commercial kit (Trinity Biotech, St. Louis). A glucose-tolerance test was performed by injecting 1.5 mg of glucose per g of body weight into the peritoneum after 6-h fasting. Blood glucose was measured through the tail tip before and after injection at the time course indicated by using the OneTouch (LifeScan, Milpitas, CA) glucose-monitoring system. ITT was conducted similarly by injecting 2 units of insulin per kg of body weight. Insulin and Acrp30 were measured by using radioimmunoassay kits (Linco, St. Charles, MO). Methods for in vitro functional assays and hyperinsulinemic–euglycemic clamp are described in Supporting Methods, which is published as supporting information on the PNAS web site. Statistics were performed by using Student’s t test. Values were presented as means ± SEMs. Significance was established at P < 0.05.

Gene Expression Analysis. Tissue RNAs were isolated by using TRIzol reagent (Invitrogen). For DNA array analysis, RNA samples from three mice of each group were amplified and hybridized to GeneChip 430A 2.0 (Affymetrix). Target genes were determined by using both ANOVA (P < 0.05) and fold-change (>1.4-fold) with the assist of GENESPRING software (Silicon Genetics, Redwood City, CA) and the BULLFROG program (34). The results were confirmed by either Northern blot analysis or Q-PCR. For Q-PCR, 1 μg of RNA was DNA-free treated, reverse-transcribed by using oligo-dT (Superscript II kit, Invitrogen), and then treated with RNAs. Samples were run in triplicate by using SYBR green (Applied Biosystems) and compared with levels of 36B4 as a control. The 0.58-kb (promoter I) and 1.3-kb (promoter II) human ACCδ promoters were generated by PCR according to the published sequence (29) and cloned into the pGL-3 basic vector (Promega). These constructs, together with expression vectors of PPARδ and RXRa, were transfected into HepG2 cells in the presence of 0.1 μM dexamethasone.

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Supporting Fig. 1. Statistical analyses of the respiratory exchange ratio (RER) of wild type (□) and PPARδ −/− mice (■) on normal chow (A) and the RER of db/db mice given either vehicle (DMSO, □) or a PPARδ agonist GW501516 (■) for 2 weeks (B). The average RER of all time points for each experiment was shown of the right. *p<0.05
Supporting Fig. 2. Weight gain and the RER of wild type and PPARδ -/- mice after high fat feeding (A) Rate of weight gain of wild type (○) and PPARδ null mice (■) under a high fat, high carbohydrate diet in a 10-week period. Insert: Body weight after ligand treatment. Four mice from each group were given either vehicle (DMSO, ○) or a PPARδ agonist GW501516 (■) for 2 weeks. There was no statistical difference in body weight before and after ligand treatment in all groups. (B) The RER of wild type and PPARδ -/- mice after high fat diet feeding. After ligand treatment, mice were placed in metabolic cages to determine the RER. There was no statistical difference between groups.
**Supporting Fig. 3.** Activation of PPARδ up-regulates genes involved in fatty acid synthesis in liver. (A) Northern blot analysis showing representative PPARδ target genes in liver. Relative expression levels were presented as numbers after quantitation, which have been normalized to the actin signal. Acetyl-CoA oxidase (AOX) was a known PPARα target in the liver and was included as a negative control. (B) Q-PCR showing relative expression levels of representative PPARδ target genes in livers of both db/db and high fat diet-induced obese (HF) mice. *P<0.05.
Supporting Fig. 4. Q-PCR analyses demonstrating that PPARδ regulates CD36 and CPT1 in a tissue-specific manner. RNA samples were isolated from liver (A), white adipose tissue (WAT) (B) and muscle (C) of vehicle and ligand (GW1516) treated db/db mice (n=3). Ligand treatment specifically induced the expression of CD36 in liver and CPT1 in muscle. The levels of known PPARα target genes such as CPT1 in liver and PPARγ target genes such as GLUT4, CD36 and TNFα in WAT were unaffected, indicating GW1516 did not cross-react with other PPAR members at the dosage used. Genes analyzed are indicated on the top. *P<0.05.