Adipose Tissue Integrity as a Prerequisite for Systemic Energy Balance

A CRITICAL ROLE FOR Peroxisome Proliferator-ActiVAted Receptor γ "\[\text{PPAR}_{\gamma}\]"

Silvia I. Anghel, Elodie Bedu, Celine Delucingue Vivier, Patrick Descombes, Béatrice Desvergne, and Walter Wahli

From the 1Center for Integrative Genomics, National Research Center Frontiers in Genetics, University of Lausanne, Génopode Bldg., CH-1015 Lausanne, Switzerland and the 2Genomics Platform, National Research Center Frontiers in Genetics, Geneva University Medical School, CH-1211 Geneva, Switzerland

Peroxisome proliferator-activated receptor γ (PPARγ) is an essential regulator of adipocyte differentiation, maintenance, and survival. Deregulations of its functions are associated with metabolic diseases. We show here that deletion of one PPARγ allele not only affected lipid storage but, more surprisingly, also the expression of genes involved in glucose uptake and utilization, the pentose phosphate pathway, fatty acid synthesis, lipolysis, and glycerol export as well as in IR/IGF-1 signaling. These deregulations led to reduced circulating adiponectin levels and an energy crisis in the WAT, reflected in a decrease to nearly half of its intracellular ATP content. In addition, there was a decrease in the metabolic rate and physical activity of the PPARγ\(+/-\) mice, which was abolished by thiazolidinedione treatment, thereby linking regulation of the metabolic rate and physical activity to PPARγ. It is likely that the PPARγ\(+/-\) phenotype was due to the observed WAT dysfunction, since the gene expression profiles associated with metabolic pathways were not affected either in the liver or the skeletal muscle. These findings highlight novel roles of PPARγ in the adipose tissue and underscore the multifaceted action of this receptor in the functional fine tuning of a tissue that is crucial for maintaining the organism in good health.

White adipose tissue (WAT)\(^2\) plays a dual role in regulating energy homeostasis (1). First, it is a tissue that responds to nutrient intake by storing excess energy in the form of triglycerides (TG) and to metabolic demands associated with fasting or exercise by releasing the stored TG as free fatty acids (FFAs) and glycerol (2). Second, WAT is an endocrine organ in addition to its energy reserve functions. In fact, it integrates metabolic signals and secretes molecules, called adipokines, which in turn impact on multiple target organs, such as the liver, muscle, or brain. Therefore, it contributes significantly to the control of whole body energy homeostasis (3–5).

Deregulation of WAT functions in obesity or lipodystrophy is often linked to metabolic disorders, such as dyslipidemia, atherosclerosis, hypertension, insulin resistance, glucose intolerance, and prothrombotic and proinflammatory states (6–10). Thus, WAT functional integrity is required for the balanced body metabolism of a healthy organism.

PPARγ (NR1C3) is highly expressed in the WAT, where it plays an important role in adipogenesis and in lipid metabolism (11–13). Suppression of PPARγ expression in preadipocytes impairs their differentiation (14, 15). Furthermore, specific deletion of PPARγ in mature adipocytes causes their death, accompanied by macrophage infiltration in the affected WAT (16). In humans, heterozygous PPARγ mutations are responsible for partial lipodystrophy, severe insulin resistance, steatosis, and hypertension (17–21). The activation of PPARγ improves insulin sensitivity in both humans and mice. Agonists of PPARγ, such as the thiazolidinedione Pioglitazone, are used clinically and are effective in reducing hyperglycemia, hyperinsulinemia, and hyperlipidemia in patients suffering from type 2 diabetes (22–24). Together, these facts underline the functions of PPARγ in adipocyte differentiation and survival and underscore its role in WAT integrity and whole body homeostasis.

Although the homozygous deletion of PPARγ in a mouse model was shown to be embryonic lethal, the survival of PPARγ\(+/-\) mice by inactivation of PPARγ in all tissues except the trophoblasts was successful. These animals suffered from lipodystrophy, insulin resistance, and hypotension (25). However, deletion of only one PPARγ allele had some intriguing effects (15, 26). In fact, PPARγ\(+/-\) mice were resistant to obesity induced by a high fat diet (HFD) and, under these conditions, remained more sensitive to insulin than their WT counterparts (27). Decreased PPARγ activity under HFD conditions had a positive outcome on the development of obesity and diabetes. Based on these observations, a novel approach in type 2 diabetes therapy would include the use of PPARγ antagonists, potentially with fewer side effects compared with the present day synthetic agonists (thiazolidinediones).

Taking advantage of our PPARγ\(+/-\) mouse model, we aimed at understanding how deletion of one allele of PPARγ, which

---

1. This work was supported by Swiss National Science Foundation Grants 31000-11340411 and 3100A0-108295 (to W.W. and B.D., respectively) and by the Etat de Vaud. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
2. The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3.
3. To whom correspondence should be addressed. Tel.: 41-21-692-4110; Fax: 41-21-692-4115; E-mail: walter.wahli@unil.ch.
4. The abbreviations used are: WAT, white adipose tissue; FFA, free fatty acid; HFD, high fat diet; IGF-1, insulin growth factor 1; WT, wild type; PPARγ, peroxisome proliferator-activated receptor γ; RER, respiratory exchange ratio; SD, standard diet; TG, triglyceride(s); qRT-PCR, quantitative real-time PCR.
significantly reduces the activity of the receptor via a gene dosage effect, would affect WAT function and whole body metabolism, when the mice are fed with a standard diet (SD), a condition which does not exacerbate the lipid storage function of the WAT. The results reported herein show that deletion of one PPARγ allele affects specifically the expression of genes associated with metabolic pathways in the WAT. In addition to genes involved in lipid storage, genes involved in glycolysis, de novo fatty acid synthesis, and lipolysis were also down-regulated in the PPARγ heterozygous mice, creating a strong energy deficit in these animals. These defects in WAT functions correlated with a lowering of the metabolic rate of the whole body and were accompanied by a reduction in physical activity. These results cast doubt on a potential long term use of PPARγ antagonists for the treatment of type 2 diabetes.

EXPERIMENTAL PROCEDURES

In Vivo Animal Study—WT and PPARγ+/- male mice, of a mixed background Sv129/C56BL/6, were maintained at 23 °C on a 12-h light-dark cycle. The animals studied were between 10 and 12 weeks of age. They had free access to water and to an SD, except during fasting, when they had free access to food only, food being withdrawn for 24 h. In some experiments, 5–6-week-old animals were fed with an SD containing 0.004% of Pioglitazone (w/w) for 5 weeks. The Pioglitazone treatment protocol was adapted from Ref. 15, a study that involved PPARγ+/- animals too. Pioglitazone was kindly provided by Takeda Chemical Industries (Switzerland). The standard food pellets containing the Pioglitazone as well as the control pellets were produced by Provimi-Kliba (Switzerland). For analysis, the animals were killed in the morning between 9 and 11 a.m. by cervical dislocation, and tissues were rapidly frozen in liquid nitrogen. The animal experimentation protocols were approved by the Commission de Surveillance de l’Expérimentation Animale of the Canton de Vaud (Switzerland).

RNA Preparation—The RNA from epidydymal WAT, gastrocnemius skeletal muscle, and liver was extracted from the frozen tissues using the Trizol reagent (Invitrogen) according to the manufacturer’s instructions. The RNA for microarray analyses was further purified using Qiagen RNaseasy columns (Qiagen). The RNA quality was assessed by capillary electrophoresis on a 2100 Bioanalyzer (Agilent Technologies).

Microarray Experiment and Data Processing—To minimize interindividual variation due to the mixed background of the mouse strain, each PPARγ+/- animal had a WT counterpart coming from the same litter.

Three independent sets of total RNA samples (three WT and three PPARγ+/- animals) from epidydymal WAT and gastrocnemius skeletal muscle were isolated. cRNA was synthesized from 5 μg of total RNA, according to Ref. 71. After purification using a Qiagen RNaseasy column, aliquots of 20 μg of cRNA were fragmented. Each fragmented cRNA (15 μg) was then hybridized to an Affymetrix “Mouse Genome 430 2.0 Array” GeneChip microarray. Hybridization, washing, and scanning were according to Affymetrix instructions.

Data from the scanned chips were analyzed using the Affymetrix MAS 5.0 software (28, 29). To identify differentially expressed transcripts, pairwise comparison analyses were carried out. Each experimental sample was compared with each reference sample, resulting in nine pairwise comparisons. Transcripts were considered to be differentially expressed if their levels changed in the same direction in seven of nine comparisons. Further data filtering and analyses were performed with the Genespring (Agilent) and the Ingenuity Pathway Analysis 4.0 software.

Quantitative RT-PCR—Single-stranded cDNA templates for quantitative real time (qRT)-PCR analysis were synthesized using Superscript II reverse transcriptase and random priming, starting from the same RNAs used for the microarray analysis, and from additional independent experiments as described above. Amplicons were designed using the Primer Express software (Applied Biosystems), and their sequences were checked by BLAST against the mouse genome to ensure that they were specific for the gene being assayed. The efficiency of each primer pair was tested in a cDNA dilution series. The list of primers is available on demand.

Real time PCR was carried out in optical 384-well plates and labeled by using the SYBR green master mix (Applied Biosystems), and the fluorescence was quantified with a 7900HT SDS system (Applied Biosystems). The relative expression level of target genes was normalized according to geNorm, using β-actin, tubulin α2, and hypoxanthine guanine phosphoribosyltransferase 1 as references to determine the normalization factor (30). Fold changes were calculated from the ratio of means of the normalized quantities and their statistical significance was determined by a paired Student’s t test.

ATP Level Measurements—Frozen WAT homogenate was transferred into a plastic tube containing 6% HClO4. Following centrifugation, the supernatant was recovered and neutralized with 5.5 M KOH. The ATP concentration was measured with an ATP determination kit, a time-stable assay from Biaffin GmbH&Co KG (Germany). The kit allows quantitative determination of small amounts of ATP by a bioluminescence assay involving the oxidation of the firefly luciferase depending on the ATP present in the extracts. The ATP concentration was derived according to the manufacturer’s instructions.

Glycerol Level Measurements—The glycerol content was measured with a glycerol measuring kit (Randox). Briefly, the glycerol present in the samples was converted into a colored product measured at a wavelength of 520 nm. The glycerol concentration was then determined according to the manufacturer’s instructions.

Metabolic Measurements—Metabolic cage studies were conducted in a comprehensive laboratory animal monitoring System (8-chamber CLAMS system; Columbus Instruments, Columbus, OH). The mice were adapted to powdered food for 24 h before they were introduced into the metabolic cages, where a 48-h acclimation preceded the 24-h recording time. Information was collected on the metabolic activity, food intake, water drinking, and physical activity.

Blood was collected from the orbital sinus between 9:00 and 11:00 a.m., using heparinized microcapillary tubes and immediately centrifuged. The serum fraction was frozen immediately. Depending on the experiment, the animals were either normally fed or fasted for 24 h.
The plasma concentrations of TG, free fatty acids (FFAs), glycerol, and ketone bodies were measured at the Mouse Clinic Institute (ICS; Strasbourg, France) on a Olympus AU-400 automated laboratory work station (Olympus-SA France) using commercial reagents (Olympus Diagnostica GmbH, Lismeehan, Ireland).

The plasma leptin and adiponectin concentrations were measured using the mouse leptin enzyme-linked immunosorbent assay kit and the mouse adiponectin enzyme-linked immunosorbent assay (Linco Reserach).

The plasma glucose levels were measured with an Accu-Chek Sensor glucometer (Roche Applied Science), and the plasma insulin concentrations were measured with an Ultra mouse insulin enzyme-linked immunosorbent assay kit (Mercodia SA).

RESULTS

Decreased Metabolic Rate in PPARγ−/− Mice—In PPARγ−/− animals, PPARγ mRNA and protein (PPARγ1 and PPARγ2) levels were reduced by half compared with those of WT mice (31). This prompted us to explore the impact of this reduced PPARγ expression on whole body metabolism in the absence of any excess energy challenge, as is usually done with HFD feeding in assessing the role of PPARγ in lipid storage. Instead, the PPARγ−/− mice were fed with an SD. Metabolic parameters of the PPARγ−/− mice and their WT littermates were determined using metabolic cages. As expected, both mutated and WT animals consumed more O2 and produced more CO2 during the dark cycle, when they are generally more active (Fig. 1A, left). Although the PPARγ−/− mice had a similar weight (Table S1) and ate an equal amount of food (data not shown), they consumed less oxygen and produced less CO2 during both the light and dark cycles when compared with their WT counterparts, a difference reflected in a decrease of 14% in the metabolic rate (heat production) of PPARγ−/− animals (Fig. 1A). This effect was clearly PPARγ-dependent, since a 5-week treatment with SD containing the PPARγ agonist Pioglitazone, at 0.004%, alleviated the metabolic rate difference between the two genotypes (Fig. 1A, right). Moreover, there was a trend, not statistically significant, for increased O2 consumption, CO2 production, and a higher metabolic rate in Pioglitazone-treated PPARγ−/− mice, whereas such a tendency was not observed in WT animals.

Deletion of One PPARγ Allele Does Not Affect the Carbohydrate to Lipid Ratio in Metabolic Fuel Utilization—To determine whether the decrease in the metabolic rate of PPARγ−/− animals was associated with alterations in the use of carbohydrates versus lipids as fuel molecules, we calculated the

![Figure 1. Metabolic rate, fuel consumption, and total physical activity in PPARγ−/− and control mice.](image-url)
respiratory exchange ratio (RER). The RER (equal to VO₂/VCO₂) indicates whether lipids (RER = 0.7) or carbohydrates (RER = 1.0) are being oxidized to produce energy. Both genotypes consumed carbohydrates as the main energy source (Fig. 1B, left). This result disagrees with the notion of a metabolic compensation through increased fat oxidation in PPARγ+/- mice (9). Moreover, the Pioglitazone treatment had no significant effect on the choice of fuel type (Fig. 1B, right).

**Decreased Physical Activity in PPARγ+/- Mice**—Since a decreased metabolic rate in the mutated animals may correlate with a change in behavior, we measured their physical activity (horizontal and rearing movements). Interestingly, the PPARγ+/- mice presented a 23% decrease in total activity (Fig. 1C, left). This phenotype correlated with a strong decrease in the plasma adiponectin concentration in PPARγ+/- mice, whereas the leptin level remained unchanged (Figs. 2A and S1). This observation is in agreement with the reduced spontaneous motor activity of transgenic mice overexpressing an antisense adiponectin RNA, resulting in decreased circulating adiponecin levels (32). The Pioglitazone treatment corrected this decrease in physical activity, suggesting an implication of PPARγ (Fig. 1C, right). In brief, reduced PPARγ levels decreased the metabolic rate and the physical activity of mice without changing their fuel preference.

**PPARγ+/- and WT Mice Have Similar Plasma Insulin and Glucose Profiles**—Since a decreased metabolic rate might impact on glucose and lipid homeostasis, we analyzed the plasma profile of the WT and PPARγ+/- mice. The plasma insulin concentration was normal in unchallenged animals and was decreased after a 24-h fast as expected, but no difference was observed between WT and mutant mice (Fig. 2B). Moreover, the glycemia was also normal in PPARγ+/- mice, which however had a significantly attenuated response to fasting (Fig. 2C). In fact, the fasting glycemia was higher in the PPARγ+/- mice compared with that of the WT animals. Fasting for 24 h decreased the glucose level by 45% in WT mice, whereas it was decreased by only 30% in the PPARγ+/- animals. Thus, after fasting, PPARγ+/- mice presented a less pronounced hypoglycemia.

**The Plasma Lipid Profile of PPARγ+/- Mice Reveals an Alteration in Lipolytic Activity**—In fed conditions, the plasma FFA concentrations were normal, and no deregulation was observed in PPARγ+/- mice (Fig. 2D). WT animals responded normally to fasting by liberating FFAs from the WAT into the circulation, thus increasing the plasma FFA concentration. Remarkably, no significant increase was observed in the PPARγ+/- mice, suggesting a deregulation of the lipolytic activity of the PPARγ+/- WAT. This defect was confirmed by measuring the circulating glycerol concentration. As for the FFAs, there was no difference in the fed glycerol concentration between WT and PPARγ+/- mice (Fig. 2E). However, the PPARγ+/- mice responded less well to fasting, since they increased their plasma glycerol concentration by only 32%, compared with the 62% monitored in WT mice. Given that the fasting glycerol and FFA concentrations are indicators of the lipolytic activity in the WAT, we concluded that PPARγ+/- mice might have a decreased lipolytic activity. This alteration should also be detectable in the WAT itself, in which the total glycerol (glycerol + glycerol-3-P) originates from glycolysis, glyceroenogenesis, and lipolysis. There was a 23% decrease in total glycerol content of the PPARγ+/- WAT, suggesting that at least one of the three above functions or all of them were impaired (Fig. 3).

Reduced circulating FFA levels should have consequences for ketone body synthesis in the liver, which depends on FFA availability. We measured the ketone body concentrations after fasting in both WT and PPARγ+/- mice (Fig. 2F). PPARγ+/- mice were less efficient in producing ketone bodies, since their plasma concentration of this peripheral organ fuel was 33% lower than in WT animals. Thus, this decreased supply in ketone bodies might reflect the reduced availability of FFAs in PPARγ+/- mice. The TG concentrations were increased by 38% after fasting in WT mice, which reflects the recycling to TG-very low density lipoprotein by the liver of a portion of the FFA liberated by the WAT during fasting (Fig. 2G). In agree-
PPARγ Controls WAT Integrity and Metabolic Rate

ment with the observations reported above, the plasma TG concentration was not increased in PPARγ+/− mice, in contrast to that measured in WT animals. The reason why fed PPARγ+/− animals also presented reduced ketone body levels remains to be elucidated (Fig. 2F).

Based on the results described so far, we hypothesized that the PPARγ+/− mice decreased their metabolic rate and their physical activity to adapt to a diminished energy supply. Three organs, the liver, skeletal muscle, and WAT, are primarily involved in energy supply and consumption. Deletion of one PPARγ allele might have affected the expression pattern of PPARγ target genes. This possibility was tested by assessing the expression of genes involved in metabolic pathways of the three key organs mentioned above by microarray analysis (WAT and skeletal muscle) and/or qRT-PCR (WAT and liver). The expression of genes not represented in the microarray was analyzed by qRT-PCR.

The Expression of Metabolic Genes Is Not Affected in the Liver of PPARγ+/− Mice—The liver is one of the major organs responsible for whole body energy balance. PPARγ is expressed at low levels in the liver under normal conditions but is increased in steatosis induced by HFD or other pathophysiological conditions (11–13). The expression pattern of several metabolic genes, which are known to be transcriptionally regulated by PPARs, was tested by qRT-PCR. We found that the expression in the liver of the metabolic genes listed in Table 1 was not affected in the PPARγ+/− animals fed an SD. In fact, genes involved in the glycolytic or FA oxidation pathways, such as very long chain acyl-CoA dehydrogenase, long chain acyl-CoA dehydrogenase, and carnitine palmitoyltransferase 1, were expressed at comparable levels in PPARγ+/− and WT mice. Similarly, the gluconeogenic pathway was not affected, even during fasting (Fig. S2). In particular, known PPAR target genes, such as those encoding phosphoenolpyruvate carboxykinase, glycerol-3-phosphate dehydrogenase 1, glyceraldehyde kinase, aquaporin 3, and glucose-6-phosphatase, were not deregulated. Since PPARγ is a regulator of these genes in liver, these results suggest that the PPARγ insufficiency did not induce a compensatory activation of PPARδ. In addition, these results are in agreement with the RER measurements (Fig. 1B, left), which showed no fuel source switch between PPARγ+/− and WT mice. We concluded that the liver of PPARγ+/− mice on SD is not the organ primarily responsible for the deficiency in energy supply, the lowering of the metabolic rate, and the reduction in motor activity of these animals.

FIGURE 2. Plasma profile of PPARγ+/− and control mice. A, plasma adiponectin concentrations; B, plasma insulin concentrations; C, plasma glucose concentrations; D, FFA plasma levels; E, plasma glycerol concentrations; F, plasma ketone body concentrations; G, plasma TG concentrations. A, fed WT, n = 11; fed PPARγ+/−, n = 12. B–G, fed WT, n = 7; fasted WT, n = 11; fed PPARγ+/−, n = 6; fasted PPARγ+/−, n = 6. Values are expressed as mean ± S.E.; *, p ≤ 0.05.
The Expression of Metabolic Genes Is Not Affected in Skeletal Muscle of PPARγ<sup>+/−</sup> Mice—The skeletal muscle is a key site of energy consumption. PPARγ is weakly expressed in this organ, and its selective deletion in skeletal muscle can affect insulin sensitivity of the liver (33) as well as muscle and WAT insulin sensitivity (34).

In our study, deletion of one PPARγ allele did not affect the expression of metabolic genes in the gastrocnemius skeletal muscle. Large scale analysis of PPARγ<sup>+/−</sup> mice and their WT counterparts on SD showed that of the 39,000 transcripts analyzed, only a few were deregulated in the PPARγ<sup>+/−</sup> mice (data not shown), and none of those was among the metabolic genes listed in Table 1. Thus, it is worth noting that, at least at the mRNA expression level, there was no deregulation of the expression of glycolysis, glycolysis, and FA oxidation in the PPARγ<sup>+/−</sup> mice. In conclusion, the skeletal muscle is apparently not responsible for the energy deficit observed in the PPARγ<sup>+/−</sup> mice, which argues against a significant role of PPARγ in this tissue under SD conditions.

The Expression of Metabolic Genes Is Affected in PPARγ<sup>+/−</sup> WAT—Since PPARγ is expressed at high levels in WAT, it was of interest to analyze the transcriptional profile of the PPARγ<sup>+/−</sup> and WT WAT. The majority of genes deregulated in PPARγ<sup>+/−</sup> WAT were classified into two major categories. First, genes involved in cell stress, detoxification, and inflammatory pathways were up-regulated; second, genes involved in metabolic and energy production pathways were down-regulated (Fig. 4). The present study concentrated on the second category only. Taken together, these results validated our hypothesis of a marked metabolic deregulation and energy production impairment in the PPARγ<sup>+/−</sup> WAT.

The Insulin and IGF-1 Signaling Pathways Are Down-regulated in the PPARγ<sup>+/−</sup> WAT—The microarray analysis revealed that the insulin as well as the insulin growth factor 1 (IGF-1) signaling pathways were down-regulated in PPARγ<sup>+/−</sup> WAT. Insulin is a key regulator of fuel metabolism, whereas IGF-1 is mostly involved in cell survival, growth, and differentiation. However, much remains to be clarified, since IGF-1 was also shown to control, at least in part, the expression of metabolic enzymes (35, 36). IGF-1 expression was down-regulated by 46% in the PPARγ<sup>+/−</sup> WAT, a result that is in agreement with our previous work (Table 2) (31). The present analysis went further by showing that not only IGF-1 expression was deregulated in PPARγ<sup>+/−</sup> WAT but also several other genes in the activation cascade downstream from IGF-1, such as c-Jun and 3- monoxygenase tryptophan 5-monoxygenase activation protein γ (14-3-3-γ), along with the insulin-like growth factor-binding protein, which were down-regulated by 39, 36,
and 46%, respectively (Table 2). Thus, processes activated by IGF-1 were affected in the mutated WAT, namely adipocyte differentiation and survival and stimulation of glucose and lipid uptake (37–40).

One major actor in the insulin pathway is the insulin receptor substrate 1; its mRNA was down-regulated by 44% in the PPARγ+/− WAT (Table 2). Furthermore, the mRNAs of the eukaryotic translation initiation factor 4E-binding protein 1, phosphodiesterase 3B, and Rap guanine nucleotide exchange factor 1 belonging to the same pathway were also down-regulated by 41, 40, and 34%, respectively. These results extend those of a previous study, which showed that the expression of phosphodiesterase 3B as well as its insulin-induced activation were decreased in the PPARγ+/− WAT (41). On the contrary, an inhibitor of the insulin pathway, called protein-tyrosine phosphatase receptor-type F was up-regulated by 2-fold in the PPARγ+/− WAT (Table 2).

Glucose Uptake and Glycolysis Are Decreased in the PPARγ+/− WAT—Gene expression changes identified by microarray and/or assessed by qRT-PCR indicated that glucose uptake, glycolysis, and its associated lipogenesis, which are controlled by insulin, were deregulated in the PPARγ+/− WAT (Table 2 and Fig. 5). The expression of the insulin-independent glucose transporter 1 was decreased by 65%, suggesting a decreased basal glucose entry into the adipocytes (Fig. 5). This effect was corrected by Pioglitazone treatment, showing the involvement of PPARγ in this deregulation (Fig S3). In contrast, the expression of glucose transporter 4 was not affected.

Once taken up by the adipocytes, glucose is processed via three main pathways. In brief, 35% of the glucose is used for TG synthesis, 50% is used for lactate production, and finally 15% is used for ATP production that provides the energy for adipocyte maintenance and survival (42). One of the main enzymes of the glycolytic pathway, hexokinase 2, was down-regulated by 40% in PPARγ+/− WAT (Table 2 and Fig. 5). Its expression was also restored by Pioglitazone treatment (Fig S3). The level of this
enzyme is stimulated by insulin via sterol regulatory element-binding protein-1c (43, 44) (Fig. 6). In line with this effect, fasting decreases and refeeding restores hexokinase 2 levels in different tissues, including WAT (44). Therefore, the decreased expression of hexokinase 2 in the PPARγ+/− WAT is consistent with impaired insulin signaling in the mutated animals.

6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase is also part of the glycolytic pathway. Four genes encode 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB1 to -4), and it is PFKFB3 that was identified as the isoform that is probably responsible for the activation of glycolysis in adipocytes. PFKFB3 was down-regulated by 40% in the PPARγ+/− WAT (Table 2). Data so far available support a role of this enzyme in TG synthesis in adipocytes (45).

Based on these combined results, we conclude that glucose uptake, as well as the glycolytic pathway, are defective in the PPARγ+/− WAT (Fig. 6), and this alteration is linked to the decreased activity of PPARγ.

De Novo Fatty Acid Synthesis Is Down-regulated in the PPARγ+/− WAT—Acetyl-CoA, produced by catabolic pathways, is used for FA synthesis (Fig. 6). Pyruvate carboxylase, whose activity increases during adipocyte differentiation, is thought to have a lipogenic function by providing amounts of acetyl groups and NADPH for fatty acid synthesis. Its gene is a direct PPARγ target in adipose tissue (46). Consistent with this finding, expression of the pyruvate carboxylase gene was decreased by 32% in the PPARγ+/− WAT. An even more pronounced down-regulation was observed for fatty acid synthase, which produces palmitate from malonyl-CoA, and for fatty acid synthase, which produces palmitate from malonyl-CoA. Their levels were reduced by 47 and 39%, respectively (Table 2 and Fig. 5), and their expression was restored by Pioglitazone treatment (Fig. S3). Interestingly and in line with our findings, the expression of these enzymes was previously shown to be increased by the PPARγ agonist GW1929 (47).

In addition to carbon supply (acetyl-CoA), the de novo production of fatty acids requires reducing power, in the form of NADPH. NADPH is generated by the oxidation of glucose 6-phosphate via an alternative pathway to glycolysis, the pentose phosphate pathway (Fig. 6). Treatment of 3T3L1 adipocytes with the PPARγ agonist rosiglitazone was shown to up-regulate the expression of enzymes involved in this pathway (48). It is regulated mainly by the activation of glucose-1-dehydrogenase, a deficiency of which results in a decrease of NADPH production (48, 49). Glucose-1-dehydrogenase expression was down-regulated by 41% in PPARγ+/− WAT. Transketolase is another enzyme linking the pentose phosphate pathway to the glycolytic pathway. Its expression was down-regulated by 49% in PPARγ+/− WAT (Table 2). Transketolase expression was shown to be decreased in insulin receptor knock-out mice, and transketolase+/− mice suffer from growth retardation and preferential reduction of the WAT (42, 50). Thus, any reduction in the expression of these two key enzymes reflects decreased FA synthesis in the PPARγ+/− WAT (Fig. 6).

Deletion of PPARγ Decreases the Expression of Enzymes Involved in Lipid Storage—It is thought that the major source of activated glycerol (glycerol-3-P) used by the WAT to esterify FA is produced by the glycolytic pathway during feeding and by the glyceroneogenetic pathway during fasting (51, 52). The production of glycerol-3-P from gluconeogenic precursors, such as amino acids and lactate, is called glyceroneogenesis, and it is one of the most important pathways in the adipocytes (53–55). Glyceroneogenesis is controlled by two enzymes, phosphoenolpyruvate carboxykinase and glycerol-3-phosphate dehydrogenase 1. Phosphoenolpyruvate carboxykinase regulation was shown to be exclusively transcriptional, with a participation of PPARγ in WAT and PPARα in the liver, through two peroxisome proliferator response element sites present in the promoter (56, 57). Glycerol-3-phosphate dehydrogenase 1 was also shown to be a PPARγ target in WAT (58). The expression of these two enzymes was decreased in the PPARγ+/− WAT, by 45 and 29%, respectively (Table 2 and Fig. 5). Although increasing their expression in both genotypes, Pioglitazone treatment was unable to completely alleviate the difference between PPARγ+/− and WT mice, suggesting an additional regulation, which may depend on the promoter context (Fig. S3) (48).

Diacylglycerol acyltransferase 1 catalyzes the terminal and only committed step in triacylglycerol esterification by using activated glycerol (glycerol-3-P) and fatty acyl-CoA as substrates. Diacylglycerol acyltransferase 1 is necessary for adipose tissue formation and essential for its survival (59). Diacylglycerol acyltransferase 1 was showed to be up-regulated by the PPARγ agonist GW1929 (47) and was consistently down-regulated by 29% in PPARγ+/− WAT.

The reduction in phosphoenolpyruvate carboxykinase, glycerol-3-phosphate dehydrogenase 1, and diacylglycerol acyltransferase 1 expression as well as the decrease in glycerol content measured in PPARγ−/− WAT are in agreement with a reduced glyceroneogenesis and lipid accumulation in the
mutant WAT. These results are in line with the role of PPARγ in lipid storage and with the smaller adipocytes, with reduced TG content found in the WAT of PPARγ+/− mice (15).
DISCUSSION

Adipose tissue integrity is crucial for whole body energy homeostasis. Its functional deregulation is usually found associated with the metabolic syndrome. Among transcription factors, PPARγ occupies key functions in WAT, where it participates in adipocyte differentiation and maintenance, including promotion of lipid storage (65). In the absence of PPARγ, preadipocytes do not differentiate into adipocytes, and its deletion in mature adipocytes leads to their death by necrosis (16).

We suspected that lipid storage is by far not the sole metabolic function of PPARγ. However, in previous studies, challenging the PPARγ+/− mice with an HFD most likely exacerbated the lipid storage function of PPARγ, and therefore other key functions went unnoticed. For this very reason, we chose to study the metabolic role of PPARγ in the absence of any specific nutritional challenge by investigating young adult PPARγ+/− male mice fed on an SD.

The plasma analyses showed that insulin and glucose concentrations were normal in basal feeding conditions, there was an alteration of the glycemic control in the mutated mice during fasting. Furthermore, the plasma lipid profile of these animals, especially FFA and glycerol, suggested the possibility of an abnormal lipolysis. This presumption was strengthened by the fact that the plasma concentrations of ketone bodies, which are produced in the liver from FFA that are released by WAT during lipolysis, were low. Although this would designate the adipose tissue as the main culprit for these deregulations, we nevertheless extended our study to the liver and skeletal muscle. All three organs are either important consumers (skeletal muscle) or producers (WAT and liver) of energy. Furthermore, all three express PPARγ, although at different levels, WAT presenting by far the highest levels of this receptor. Based on their gene expression profiles, we concluded that the liver and skeletal muscle in PPARγ+/− mice are most likely not involved in the metabolic deregulation of these animals when fed an SD. Of all of the genes tested that are involved in glycolysis and FA oxidation in both tissues, as well as in gluconeogenesis in the liver, none presented a modified expression in the PPARγ+/− mice. These results are in agreement with liver and muscle gene expression profiles in fatless mice or mice with an organ-specific deletion of PPARγ in skeletal muscle. In these studies, no modification was observed in the expression of genes involved in β-oxidation and gluconeogenesis (10, 33, 34, 66).

On the contrary, deletion of one PPARγ allele had unexpectedly profound effects on the expression of genes involved in WAT differentiation and energy production. The present study shows that genes involved in the IGF-1 signaling pathway, known to participate in adipocyte differentiation and growth (31, 37–40), were down-regulated in PPARγ+/− WAT. This explains the resistance to growth hormone action and the smaller adipocyte size already observed by us and others in the PPARγ+/− WAT (15, 31, 67). In addition to the IGF-1 pathway, genes belonging to the insulin signaling pathway, which is an important activator of the glycolytic and lipogenic pathways in WAT, were also down-regulated.

Unexpected at first sight, but in agreement with a down-regulation of the insulin pathway, basal glucose uptake and glycolysis were decreased in the mutant WAT. Glycolysis is thought to play three major roles in WAT by (i) participating in the de novo synthesis of fatty acids, (ii) promoting their storage as fat, and finally (iii) producing ATP for adipocyte survival. First, the acetyl-CoA and NADPH synthesized by the glycolytic and pentose phosphate pathways, respectively, were decreased in the PPARγ+/− WAT (Fig. 6). As an immediate consequence, de novo fatty acid synthesis was decreased, an effect reinforced by the down-regulation of enzymes involved in this process. Second, storage was altered, since glycolysis and glyceroneogenesis were affected in the PPARγ+/− WAT. Third, glucose is required for adipocyte survival, and ATP production is an important feature of this process, deficiency of which affects the integrity of the cell. We observed a significant decrease of the ATP levels in the WAT of the mutant mice. This result is in agreement with previous data from cell culture experiments, where decreased ATP concentration in 3T3L1 or primary adipocytes interfered with insulin signaling and lipolysis. Glycolysis and FA oxidation are the major ATP producers, but no increase of β-oxidation was observed in the mutant WAT to compensate for the energy deficit. Moreover, genes involved in the lipolytic activity as well as in the glycerol release were down-regulated in the PPARγ+/− WAT. Thus, PPARγ+/− WAT suffers from a generalized energy shortage probably due to the ATP crisis in the adipocytes, which we link to a deregulation of the glycolytic pathway. We have shown previously that tissue-specific ablation of PPARγ in adipose tissue causes the death, within a few days of the PPARγ-deficient adipocytes (16). We observed a necrosis rather than apoptosis, which triggered an inflammatory response in the affected adipose tissue (16). Usually, necrosis is caused by a metabolic disruption and ATP depletion, whereas apoptosis requires ATP without a clearly defined point of no return (68). The phenotype of the PPARγ+/− adipocytes suggests that what caused the death of PPARγ−/− adipocytes was in fact a metabolic breakdown associated with a loss of ATP.

To our knowledge and for the first time, the findings reported herein link PPARγ expression levels with the regulation of the systemic metabolic rate and physical activity. Although deletion of just one PPARγ allele decreased the metabolic rate and physical activity of the mice, we recorded no effect on the relative amounts of carbohydrates versus lipids used as fuel molecules. This observation is in agreement with the absence of deregulation in the expression of enzymes involved in FA oxidation. Evidence that this systemic effect is PPARγ-specific comes from the Pioglitazone treatment of the PPARγ+/− mice, which alleviated the deleterious effect of heterozygocity. Thus, endogenous ligands of PPARγ are most probably not abundant or not efficient enough to compensate for the lower levels of PPARγ protein by significantly increasing its activity. This observation underscores the importance of the combined effects of receptor expression level and ligand availability to reach the optimal tuning of PPARγ activity and, in turn, of its target genes, especially in WAT.

We speculate that the reduced level of both metabolic rate and physical activity are part of a protective survival strategy in conditions of energy shortage. When faced with an energy crisis, animals employ various behavioral and physiological
responses to reduce metabolism, which prolongs the period of time during which energy reserves can cover metabolic needs. Such behavioral responses can include a reduction in metabolic rate and spontaneous locomotor activity (69). It is possible that the energy crisis in WAT is signaled to the whole body by a reduction in the circulating adiponectin levels. Interestingly, some of the physiological characteristics of the PPARγ−/− mice resemble those of PPARα−/− animals during fasting, such as hypoketogenesis and reduced activity (32, 70).

Food intake itself cannot explain the metabolic and activity phenotype, since the PPARγ−/− mice ate the same amount of food and had a similar weight as their WT counterparts. Therefore, the fate of the energy spared due to the metabolic and activity down-regulation remains to be elucidated.

Collectively, the data obtained by taking advantage of PPARγ−/− animals fed an SD showed that PPARγ activity in WAT is not only important for fat storage, as previously showed. In fact, in the absence of any nutritional challenge, PPARγ controls IGF-1 and insulin signaling with effects on energy production via the regulation of the glycolytic and lipolytic pathways. Most importantly, deregulation of these functions in WAT most likely influences the whole body energy balance with its impacts on metabolic rate and physical activity, which appear to adapt to the available energy.

Acknowledgments—We are grateful to Patrick Gouaït and his team for technical help in animal handling; to the members of the genotyping laboratory, particularly to Armelle Bauderet, for help with animal characterization; and to Joel Gyger for help with the manuscript and suggestions.

REFERENCES

1. Cinti, S. (2005) Prostaglandins Leukot. Essent. Fatty Acids 73, 9–15
2. Mandrup, S., and Lane, M. D. (1997) J. Biol. Chem. 272, 5367–5370
3. Juge-Aubry, C. E., Somm, E., Giusti, V., Pernin, A., Chicheportiche, R., Verduno, C., Rohner-Jeanrenaud, F., Burger, D., Dayer, J. M., and Meier, C. A. (2003) Diabetes 52, 1104–1110
4. Fruhbeck, G., Gomez-Ambrosi, J., Muruzabal, F. I., and Burrell, M. A. (2003) Am. J. Physiol. 280, E827–E847
5. Fried, S. K., Bunkin, D. A., and Greenberg, A. S. (1998) J. Clin. Endocrinol. Metab. 83, 847–850
6. Simha, V., and Garg, A. (2006) J. Biol. Chem. 281, 4527–4534
7. Jan, V., Cervera, P., Maachi, M., Baudrimont, M., Kim, M., Vidal, H., Girard, A., Rieusset, J., Seydoux, J., Anghel, S. I., Escher, P., Michalik, L., Soon Tan, N., Vandesompele, J., De Preter, K., Pattyn, F., Van Roy, N., and Depege, F. (2002) J. Clin. Endocrinol. Metab. 87, 2784–2791
8. Domingo, P., Maachi, M., Baudrimont, M., Kim, M., Vidal, H., Girard, A., Rieusset, J., Seydoux, J., Anghel, S. I., Escher, P., Michalik, L., Soon Tan, N., Vandesompele, J., De Preter, K., Pattyn, F., Van Roy, N., and Depege, F. (2002) J. Clin. Endocrinol. Metab. 87, 2784–2791
9. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O., Willson, T. M., and Kliwer, S. A. (1995) J. Biol. Chem. 270, 12953–12956
10. Boden, G., Homko, C., Mozzoli, M., Showe, L. C., Nichols, C., and Cheung, P. (2005) Diabetes 54, 880–885
11. Duan, S. Z., Iwashchenko, C. Y., Whitesall, S. E., D’Alecy, L. G., Duquaine, D. C., Broussis, F. C., Gonzalez, F. J., Corder, M. A., Milstone, D. S., and Mortensen, R. M. (2007) J. Clin. Invest. 117, 812–822
12. Miles, P. D., Barak, Y., He, W., Evans, R. M., and Olefsky, J. M. (2000) J. Clin. Invest. 105, 287–292
13. Yamauchi, T., Kamon, I., Waki, H., Murakami, K., Motomura, K., Kojima, K., Ike, T., Kubota, N., Terauchi, Y., Tobe, K., Miki, H., Tsuda, A., Akahama, Y., Nagai, R., Kimura, S., and Kadowaki, T. (2001) J. Biol. Chem. 276, 41245–41254
14. Hubbell, E., Liu, W. M., and Mei, R. (2002) Bioinformatics 18, 1585–1592
15. Liu, W. M., Mei, R., Di, X., Ryder, T. B., Hubbell, E., Dee, S., Webster, T. A., Harrington, C. A., Ho, M. H., Baid, J., and Smeekens, S. P. (2002) Bioinformatics 18, 1593–1599
16. Vanesompelje, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., and Speleman, F. (2002) Genome Res. 12, research0034.1–research0034.11
17. Rieu, J., Seydoux, J., Anghel, S. I., Escher, P., Michalik, L., Soon Tan, N., Metzger, D., Chambon, P., Wahli, W., and Desvergne, B. (2004) Mol. Endocrinol. 18, 2363–2377
18. Saito, K., Arata, S., Hosono, T., Sano, Y., Takahashi, K., Choi-Miura, N. H., Nakano, Y., Tobe, T., and Tomita, M. (2006) Biochim. Biophys. Acta 1761, 709–716
19. Norris, A. W., Chen, L., Fisher, S. J., Szanto, I., Ristow, M., Jozsi, A. C., Willson, T. M., Gooday, L. J., Gonzalez, F. J., Spiegelman, B. M., and Kahn, C. R. (2003) J. Clin. Invest. 112, 608–618
20. Hevener, A. L., He, W., Barak, Y., Le, J., Bandyopadhyay, G., Olson, P., Wilkes, J., Evans, R. M., and Olefsky, J. (2003) Nat. Med. 9, 1491–1497
21. Allen, T., Zhang, F., Moodie, S. A., Clemens, L. E., Smith, A., Gregoire, F., Bell, A., Muscat, G. E., and Gustafson, T. A. (2006) Diabetes 55, 2523–2533
22. Frick, F., Oscarsson, J., Vikman-Adolfsson, K., Ottosson, M., Yoshida, N., and Ehn, S. (2000) Am. J. Physiol. Endocrinol. Metab. 278, E729–E737
23. Gerfault, V., Louveau, I., and Mourot, J. (1999) Gen. Comp. Endocrinol. 114, 396–404
24. Smith, P. J., Wise, L. S., Berkowitz, R., Wan, C., and Rubin, C. S. (1988) J. Biol. Chem. 263, 9402–9408
25. Di Cola, G., Cool, M. H., and Accili, D. (1997) J. Clin. Invest. 99, 2538–2544

JOURNAL OF BIOLOGICAL CHEMISTRY
VOLUME 282 • NUMBER 41 • OCTOBER 12, 2007
29956
