Peroxisome Proliferator-Activated Receptor-α Control of Lipid and Glucose Metabolism in Human White Adipocytes

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This work aimed at characterizing the role of peroxisome proliferator-activated receptors (PPAR) in human white adipocyte metabolism and at comparing PPARα and PPARγ actions in these cells. Primary cultures of human fat cells were treated with the PPARα agonist GW7647 or the PPARγ agonist rosiglitazone. Changes in gene expression were determined using DNA microarrays and quantitative RT-PCR. Western blot and metabolic studies were performed to identify the biological effects elicited by PPAR agonist treatments. GW7647 induced an up-regulation of β-oxidation gene expression and increased palmitate oxidation. Unexpectedly, glycolysis was strongly reduced at transcriptional and functional levels by GW7647 leading to a decrease in pyruvate and lactate production. Glucose oxidation was decreased. Triglyceride esterification and de novo lipogenesis were inhibited by the PPARα agonist. GW7647-induced alterations were abolished by a treatment with a PPARα antagonist. Small interfering RNA-mediated extinction of PPARα gene expression in hMADS adipocytes attenuated GW7647 induction of palmitate oxidation. Rosiglitazone had no major impact on glycolysis and β-oxidation. Altogether these results show that PPARα can selectively up-regulate β-oxidation and decrease glucose utilization in human white adipocytes. (Endocrinology 151: 123–133, 2010)
pressed in tissues with high lipid catabolic capacities (1, 7). PPARα activation increases transcription of genes that encode proteins involved in fatty acid mitochondrial uptake and β-oxidation such as carnitine palmitoyltransferase 1 (CPT1) and very-long-chain acyl-CoA dehydrogenase (ACADVL). Its role extends beyond the control of fatty acid oxidation pathway through tissue-specific functions (8). In the liver, PPARα promotes gluconeogenesis and ketone body synthesis and is involved in lipoprotein assembly. In the heart, it participates in the regulation of the metabolic switch between glucose and lipid oxidation.

PPARα is also well expressed in brown adipose tissue where, together with PGC1α, it regulates key components of the thermogenic program (7, 9). PPARα agonists, WY-14643 or fibrates, induce the transcription of the uncoupling protein-1 (UCP1) gene in brown adipocytes (10). Because of its low expression, PPARα has not been extensively investigated in white adipose tissue (WAT). However, some studies in rodents indicate that PPARα has also a role in white fat cells. In mice, the activation of β3-adrenergic receptors induces adipose tissue remodeling. In particular, expression of fatty acid catabolic enzymes and oxidative metabolism are increased, and those effects are partly mediated by PPARα (11). PPARα is also mandatory for adipose tissue disappearance in hyperleptinemic rats, which is related to the induction of WAT oxidative enzymes (12). In the WAT of PPARα knockout mice, basal lipogenic flux and its response to a cholesterol-enriched diet are altered (13). PPARα agonists up-regulate the expression of adiponectin and of one of its receptors, ADIPOR2, in 3T3-L1 cells and in mice, whereas PPARα invalidation abolishes the fibrate-induced rise in plasma adiponectin level (14, 15). Finally oleoylthanolamide enhances lipolysis only in isolated mouse adipocytes that express PPARα (16).

In human adipose tissue, PPARα is also expressed and its mRNA is negatively correlated with body mass index (1, 17). However, there is a paucity of studies regarding PPARα function, whereas extrapolation of rodent data to humans is hazardous because of noteworthy species differences. Indeed, the potency of PPARα agonists differs between the mouse and human nuclear receptor (18), and PPARα activation in the liver leads to peroxisome proliferation and hepatocarcinoma in mice but not in humans (19). Nonetheless, two studies stress the role of PPARα in human adipocytes. Using WY-14643, which is a potent and specific agonist for murine but not for human PPARα, an induction of fatty acid oxidation was observed (20, 21). Moreover, we showed that glycerol kinase expression and activity are increased in human white adipocytes treated with the selective PPARα agonist GW7647, which reflects the direct binding and activation of the glycerol kinase promoter by PPARα (22).

In the present work, we addressed the role of PPARα in the regulation of human white adipocyte metabolism. Microarray analysis of transcriptional changes induced by pharmacological activation of PPARα and PPARγ in differentiated fat cells revealed alterations of distinct sets of genes. PPARα activation enhanced gene expression of the fatty acid oxidation pathway, whereas it down-regulated glycolysis genes. Accordingly, fatty acid oxidation and glycolysis were increased and decreased, respectively. Moreover, PPARα activation diminished lipogenesis. PPARγ activation had no effect on β-oxidation and glycolysis. These results show that activation of PPARα promotes fatty acid utilization in human white adipocytes.

Materials and Methods

Materials

GW7647, GW6471, etomoxir, and AICAR (2-amino-1, (2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl[pyrrole-3-carboxamide) were purchased from Sigma-Aldrich (St. Louis, MO) and rosiglitazone (BRL49653) from Alexis Biochemicals (San Diego, CA). All chemicals were diluted in dimethylsulfoxide (DMSO) except AICAR, which was prepared in PBS.

Cell culture

Subcutaneous abdominal WAT was obtained from female subjects undergoing plastic surgery in agreement with French laws on biomedical research. Human preadipocytes from adipose tissue stromavascular fraction were differentiated as previously described (23, 24). Only cultures with more than 70% of cells differentiated into lipid droplet-containing adipocytes at d 13 were selected for further experiments. Culture medium was replaced, and cells were treated with rosiglitazone (1 μM) or GW7647 (1 μM). When used, etomoxir (1 μM) or GW6471 (10 μM) was added at the same time as PPAR agonists. Vehicle was added to control cells. After 48 h treatment, medium was collected for metabolite measurements, and cells were either harvested for RNA or protein extraction or used in metabolic assays.

hMADS cells were cultured and differentiated as previously described (25–27). For RNA interference assay, two small interfering RNA (siRNA) targeting PPARα were used independently. The targeted sequences are for PPARα 5′-GGATAGTTCTG-GAACCTT-3′ or 5′-GGCCUCAGGCUAUCAUUAC-3′ and for green fluorescent protein (GFP) 5′-GCCAGCAGACUUCUGAUG-3′, and 200 pmol siRNA was electroporated in 2 million cells as previously described (27). Six days after microporation, differentiated cells were treated with 1 μM PPAR agonists, and after 48 h treatment, gene expression and oxidation assays were performed.

DNA microarray analysis

We treated four independent primary cultures from different subjects, with GW7647, rosiglitazone, or DMSO. After 48 h, total RNA was extracted with RNeasy mini kit (QIAGEN, Valencia, CA). Probe labeling, array hybridization and scan, data
extraction, and preprocessing were performed as described (28). Features with a signal to background ratio strictly less than 1.5 in both colors were discarded, and arrays were mean centered and normalized with Gene Cluster (29). Differentially expressed genes between agonist and vehicle treatments were determined using significance analysis of microarray (SAM) one-class analysis with features having fewer than two missing values of four (30). Data are accessible through GEO Series accession number GSE15862. SAM-ranked gene lists were used for gene ontology analysis using gene set enrichment analysis (31). A Venn diagram built on GW7647- and rosiglitazone-regulated genes was used to list genes regulated only by GW7647, only by rosiglitazone, or by both agonists. The lists were analyzed with the PANTHER classification system to determine which biological processes were enriched (32).

Quantitative RT-PCR analysis

One microgram of total RNA was treated with deoxyribonuclease I (amplification grade; Invitrogen). Reverse transcription and real-time quantitative PCR (qPCR) (Applied Biosystems, Foster City, CA) were performed as previously described (22) (supplemental Tables 1 and 2, which is published as supplemental data on The Endocrine Society’s Journals Online web site at http://mend.endojournals.org), and 18S rRNA was used as control. Results are expressed using the ΔΔCt method.

Oxidation and esterification measurements

For palmitate oxidation assays, cells in six-well plates were incubated during 3 h in 2 ml Krebs Ringer buffer (125 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1.25 mM KH2PO4, 1.25 mM MgSO4·7H2O, 25 mM NaHCO3) containing 3% BSA, 500 mM palmitic acid (Sigma), 1 μCi [14C(U)]palmitic acid (PerkinElmer, Norwalk, CT). Medium was then transferred and acidified with 1 M sulfuric acid in closed vials containing a central well filled with benzethonium hydroxide. After 2 h incubation, wells were removed, and trapped 14CO2 was measured by liquid scintillation counting. Cells were washed and then scraped in 0.05 M NaOH, and 2DG specific activity was counted and used to determine the quantity of oxidized palmitic acid.

For glucose oxidation assays, cells in six-well plates were incubated for 1 h in the same buffer containing 2% BSA, 10 mM HEPES, 2 mM glucose, and 1 μCi [3H(U)]glucose (PerkinElmer). A 2- × 2-cm Whatman 3M paper was taped on the bottom of each well and wet with 100 μl benzethonium hydroxide. After incubation, filter-trapped 14CO2 was measured by liquid scintillation counting. Medium-trapped 14CO2 was measured, and neutral lipids were extracted as described above. Glucose incorporation into fatty acid and glycerol was assessed as follows. Neutral lipids were dried and hydrolyzed in 1 ml 0.25 N NaOH in chloroform/methanol (1:1) for 1 h at 37 C. The solution was neutralized with 500 μl 0.5 N HCl in methanol. Fatty acids and glycerol were separated by adding 1.7 ml chloroform, 860 μl water, and 1 ml chloroform/methanol (2:1). Incorporation of 14C into glycerol and fatty acids was measured by liquid scintillation counting of upper and lower phases, respectively. Specific activity was counted and used to determine the quantity of oxidized/incorporated glucose equivalent.

For oxidation assays, results were normalized to total protein content of cell extracts. Measurements were made in duplicate or triplicate.

Pyruvate and lactate concentration measurements

For medium lactate concentration determination, 5 μl sample or standard was diluted in 200 μl reaction buffer (0.4 M hydr- azide; 0.2 mM glycine; 0.5 mM nicotinamide adenine dinucleotide, pH 9.9), and variation of fluorescence emission (353/430) was measured before and 90 min after addition of rabbit muscle lactate dehydrogenase (Sigma) (33). When lactate was measured in cell lysate, 50 μl was used. Pyruvate concentrations were assessed with 25 μl sample or standard in 200 μl reaction buffer (34 mM K2HPO4 \· 23 mM NaH2PO4 \· 0.325 mM EDTA disodium; 50 μM reduced nicotinamide adenine dinucleotide, pH 7). Absorbance at 340 nm was read before and 10 min after lactate dehydrogenase addition. Measures were made in duplicate or triplicate.

2-Deoxy-D-glucose (2DG) uptake assay

The day before the assay, insulin was removed from culture medium. Cells were incubated 45 min in 1 ml buffer (140 mM NaCl, 5 mM KCl, 1 mM CaCl2, 2.5 mM MgSO4·7H2O, 25 mM NaHCO3, 20 mM HEPES, 0.2% BSA) containing appropriate drugs with or without 100 nM insulin. Then 125 μCi 2-[1,2-3H(N)]deoxy-D-glucose (PerkinElmer) was added, and incubation was maintained for exactly 10 min. Culture plates were then put on ice and rinsed with 10 ml glucose in PBS and then with PBS. Cells were scraped in 0.05 M NaOH, and 2DG uptake was measured by liquid scintillation counting of cell lysate. The results were normalized to total protein content of cell lysates and expressed relative to DMSO without insulin sample.

Western blot

Cells were lysed in 50 mM Tris (pH 8), 0.5 mM dithiothreitol and 0.1% Nonidet P-40 supplemented with antiproteases and antiphosphatases. Lysates were centrifuged at 1000 × g for 10 min at 4 C, and supernatants were resolved by 8% SDS-PAGE as previously described. Pyruvate dehydrogenase kinase 4 (PDK4) and hydroxyacyl-CoA dehydrogenase B (HADHB) antibodies were purchased from Abnoma (Taipei City, Taiwan).

Statistical analyses

The number of independent cultures used for each assay is mentioned in the figure legends. Results are presented as mean ± SEM unless otherwise indicated. All statistical analyses were performed with Wilcoxon nonparametric test on paired samples using SPSS version 17.0.

Results

PPARα and PPARγ regulate gene expression in human adipocytes

PPARα is expressed in human white adipocytes albeit at a lower level than PPARγ (supplemental Fig. 1). We investigated the effects of PPARα and PPARγ activation in human differentiated preadipocytes using DNA microarrays. SAM analysis showed that the PPARγ agonist rosiglitazone up-regulated 951 genes, whereas it down-regulated...
126 Ribet et al. PPARα Controls Metabolism in Fat Cells  

TABLE 1. RT-qPCR validation of microarray data

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Microarrays were used to determine GW7647 and rosiglitazone effects in human white adipocytes. RT-qPCR were performed to confirm microarray data. Gene names are available in supplemental Tables 1 and 2. n is the number of primary cultures used for RT-qPCR. ND, Not done.

^a–^c For each treatment, fold change was calculated relative to DMSO, and its significance was assessed by SAM and Wilcoxon tests for microarray and RT-qPCR, respectively. ^d P ≤ 0.05; ^e P ≤ 0.01; ^f P ≤ 0.001.

^d–^f Differences between GW7647- and rosiglitazone-induced fold change in RT-qPCR experiments were determined using Wilcoxon tests:

GW7647 Rosiglitazone

695 genes (false discovery rate of 5%). The PPARα agonist GW7647 up-regulated 1742 genes and down-regulated 1746 genes. A total of 1008 genes were regulated by both GW7647 and rosiglitazone. Therefore, although PPARα and PPARγ share many targets in common, our results suggest that preferential targets also exist for each nuclear receptor.

To ascertain the relevance of our microarray approach, we performed RT-qPCR on 25 genes with significantly altered expression by GW7647 treatment. Overall, RT-qPCR data were in very good agreement with microarray data (Table 1). As an illustration of genes displaying different expression profiles, RT-qPCR confirmed that FAPB4 expression is more up-regulated by rosiglitazone than by GW7647. AACS mRNA was significantly down-regulated by both agonists. GK, a specific PPARα target gene, was up-regulated to a larger extent by GW7647 than by rosiglitazone.

Fatty acid oxidation and glycolysis genes are differentially regulated by PPARα and PPARγ agonists

To define whether PPAR activation altered specific cellular functions, we analyzed the ontology of GW7647- and rosiglitazone-regulated genes. Gene set enrichment analysis showed that the PPAR signaling pathway gene set was enriched for GW7647- and rosiglitazone-up-regulated genes as expected. The PANTHER classification system pointed to genes of lipid metabolism-related pathways as a set of genes regulated by both agonists as well as sets of genes regulated only by GW7647 or by rosiglitazone. Among the genes regulated only by GW7647 were genes involved in glycolysis. Therefore, we focused the functional analysis of microarray data on fatty acid and glucose metabolism pathways.

As shown in Fig. 1A, PPARα activation up-regulated four major genes involved in fatty acid oxidation. Indeed, CPT1B is the enzyme controlling fatty acyl-CoA entry into mitochondria for β-oxidation, HADHA and HADHB genes encode units forming the mitochondrial trifunctional protein, and ACA2 gene encodes a protein catalyzing the last step of β-oxidation. Surprisingly, the PPARγ agonist also increased expression of these enzymes (Table 1 and Fig. 1A). However, the increase in CPT1B and HADHB induced by rosiglitazone was significantly lower than that induced by GW7647 (Table 1 and Fig.
we measured glycolysis end product concentrations in culture medium. After 48 h PPAR agonist treatment, both pyruvate and lactate concentrations were reduced with GW7647 by 60%. Rosiglitazone slightly reduced pyruvate concentration and did not modify lactate concentration (Fig. 3, A and B). Intracellular pyruvate concentrations were too low to be acutely quantified. However, intracellular lactate concentrations exhibited similar alterations as medium concentrations (data not shown).

Glycolysis depends directly on cellular glucose uptake. The GW7647-induced down-regulation of GLUT4 mRNA suggested that this pathway may be altered (Table 1, SLC2A4). After 48 h treatment, basal glucose uptake was not altered by PPARα or PPARγ agonist, whereas it was strongly stimulated by insulin (data not shown). GW7647 reduced insulin-stimulated glucose uptake, whereas rosiglitazone had no effect (Fig. 3C). The GW7647-mediated decrease of insulin-stimulated glucose uptake was observed using 1, 10, and 100 nM insulin (data not shown).

Next, we measured glucose oxidation because it is the ultimate catabolic fate of the glycolytic product pyruvate after its conversion to acetyl-CoA by the pyruvate dehydrogenase complex (PDC). GW7647 and rosiglitazone significantly reduced glucose oxidation (Fig. 3D). The PDK4 is a glucose metabolism key regulatory step because it inactivates the PDC and appeared up-regulated by PPARα and PPARγ agonists in microarray data (Table 1). We confirmed that GW7647 and rosiglitazone increased PDK4 mRNA and protein levels (Fig. 3, E and F).

GW7647 effects on β-oxidation and glycolysis are independent

Etomoxir treatment inhibited palmitate oxidation similarly in PPAR agonist- or vehicle-treated cells (Fig. 2D). Lactate and pyruvate concentrations were unaffected by the lack of β-oxidation and were diminished 2-fold with GW7647 as in fatty acid-oxidizing cells (supplemental Fig. 2 and data not shown). Thus, the GW7647-induced increase in β-oxidation is not required for reduced glycolytic flux in human adipocytes.

GW7647 inhibits lipogenesis in human adipocytes

PPARα activation significantly altered fatty acid and glucose catabolism in adipocytes. We measured exoge-
nous fatty acid esterification and de novo lipogenesis in an attempt to determine whether GW7647 also modified anabolic pathways. GW7647 significantly reduced palmitate incorporation into triglycerides, indicating that PPARα agonist decreases fatty acid esterification (Fig. 4A). Incubating adipocytes with oleic acid resulted in the same incorporation profile (data not shown).

Glucose carbon incorporation into the glycerol and fatty acid moieties of lipids was measured. With GW7647, 40% less fatty acid was synthesized, showing that de novo lipogenesis is reduced (Fig. 4B). GW7647 had no effect on glycerol synthesis (Fig. 4C).

GW7647-induced metabolic alterations are mediated by PPARα
To verify that GW7647 alters fatty acid oxidation and glycolysis by a PPARα-dependent mechanism, we used GW6471, a PPARα antagonist (34). Basal levels of palmitate oxidation and medium lactate were unchanged by PPARα blockade (Fig. 5, A and B). When GW6471 and GW7647 were combined, the PPARα-induced decrease in lactate levels was abolished and the PPARα-induced increase of palmitate oxidation was partially inhibited (Fig. 5, A and B). The PPARα antagonist had no effect on rosiglitazone-treated cells.

To investigate the consequence of PPARα knockdown on GW7647 effects, we used another human adipocyte model. hMADS cells are stem cells derived from infant adipose tissue that steadily differentiate into functional adipocytes (25, 26). This cell line is amenable to reproducible and efficient siRNA-mediated knockdown, which proves difficult in differentiated primary adipocytes (27). PPARα silencing decreased PPARα mRNA expression by 70% (Fig. 5C). As in primary adipocytes, GW7647 up-regulated palmitate oxidation in hMADS cells (Fig. 5D). PPARα silencing strongly reduced basal and GW7647-enhanced palmitate oxidation (Fig. 5D).

Discussion
We investigated the role of PPARα in the control of human adipocyte metabolism and compared the effects of PPARα and PPARγ activation. As previously reported, PPARα and PPARγ share many target genes (35). However, they also independently alter specific sets of genes. PPARα activation with GW7647 regulated the expression of genes of the glucose and fatty acid metabolism pathways. GW7647 strongly increased palmitate β-oxidation by a PPARα-dependent mechanism. In parallel, GW7647 triggered a robust decrease in pyruvate and lactate concentrations and repressed glucose uptake and oxidation, de novo lipogenesis, and fatty acid esterification.

Because, in vivo, TZD induce an up-regulation in expression of lipid storage genes and an increase in sc adipose tissue mass, rosiglitazone-induced changes were expected (36). At the transcriptional level, we observed an up-regulation of known targets such as FABP4 and PDK4 (2, 37). A hypothesis for PPARγ antidiabetic action is the induction of a white to brown-like adipocyte shift associated with mitochondrial biogenesis and enhanced lipid oxidation capacity (38, 39). Accordingly, Bogacka and colleagues (21) reported that PPARγ agonists increased fat oxidation in differentiated human adipocytes. We noticed a slight increase in CO2 production after 48 h, but it was not statistically significant. Thus, in agreement with our previous study, we did not detect any robust induction in palmitate oxidation of rosiglitazone-treated human adipocytes (23). Differences between our results and those of others in this regard could be explained by differences in treatment length, timing, and/or culture conditions. Rosiglitazone did not alter glucose uptake with or without insulin stimulation, which was in line with the unaltered GLUT4 mRNA level. This observation is apparently not...
in agreement with the known TZD ability to alleviate the PPARγ-mediated repression of GLUT4 promoter in rat primary adipocytes (40). However, it has also been shown that rosiglitazone does not change GLUT4 expression in differentiated 3T3-L1 adipocytes, whereas it increases GLUT4 expression when added during the differentiation (41). In this study, rosiglitazone had moderate effects at the metabolic level, which is surprising in view of rosiglitazone’s multiple effects in vivo. Nonetheless, TZD in vivo action is mediated by the differentiation of new adipocytes as well as the shrinkage of mature adipocytes, which reflect PPARγ role in adipocyte differentiation and normal function maintenance, respectively (42, 43). In our model, adipocytes were already differentiated and were exposed to rosiglitazone only for 48 h, which was likely not sufficient to recapitulate in vivo PPARγ effects on adipocyte functions.

Human white adipocytes treated with a PPARα agonist showed numerous significant alterations of gene expression. We showed that PPARα activation strongly decreased lactate and pyruvate concentrations, which can result from three mechanisms: less glucose entry, glycolysis inhibition, and enhanced pyruvate utilization (supplemental Fig. 3). PPARα agonist slightly decreased insulin-stimulated glucose uptake but not to an extent that can explain the lower pyruvate and lactate concentrations. Glucose oxidation was slightly diminished, which indicated that less pyruvate was catabolized through the Krebs cycle, suggesting a lesser pyruvate inflow or a Krebs cycle slowdown. Glucose incorporation into fatty acids was decreased by GW7647, whereas its incorporation into glycerol was unchanged. Therefore, the main pyruvate catabolic pathways were not increased and the fall in pyruvate and lactate concentrations is chiefly explained by a PPARα-induced inhibition of glycolysis, which was in line with the transcriptomic analysis. Indeed, we showed a coordinate PPARα-induced down-regulation of glycolytic gene expression, whereas microarray data indicated that PPARα did not alter the gene expression of the Krebs cycle and respiratory chain enzymes. The involvement of PPARα in glycolysis is also suggested by data from PPARα−/− mouse isolated adipocytes, which produce higher lactate levels and oxidize more glucose compared with wild-type adipocytes (44). Despite a strong inhibition of GLUT4 expression, we showed only a slight reduction in insulin-stimulated glucose transport. Indeed, glucose uptake is mainly controlled by intracellular GLUT4 cycling and translocation to the plasma membrane (45).

In addition to glycolysis inhibition, our results show that GW7647 doubled β-oxidation in agreement with the up-regulation of CPT1B and fatty acid oxidation enzymes. CPT1 is a classical PPARα target gene. PPARα induces its expression in liver, heart, and skeletal muscle (7, 46). In human adipocytes, CPT1B is also a PPARα target as shown in mouse adipose tissue (11, 12). The PPARγ-induced CPT1B up-regulation is puzzling because PPARγ is promoting lipid storage. However, this regulation has been reported in adipocytes from genetically obese mice treated with rosiglitazone (36). Also in human differentiated adipocytes, three different PPARγ agonists were shown to increase CPT1B mRNA (21, 47). Consistent with their respective species-related PPARα activation potencies, GW7647 enabled a greater induction of lipid oxidation after 48 h treatment than Wy-14643 (21). PPARα activation altered lipid as well as glucose metab-
olism in human adipocyte, which is reminiscent of a PPAR role in other organs (8). Indeed, PPAR activates fatty acid oxidation in line with gene expression profiling when specifically overexpressed in mouse heart or skeletal muscle (46, 48). The cardiac-specific overexpression is accompanied by a decrease in glucose oxidation, whereas skeletal muscle-specific PPAR activation leads to whole-body glucose intolerance (46, 48). Interestingly, Finck and colleagues (48) hypothesized that PPAR-induced fatty acid oxidation leads to ATP accumulation in skeletal muscle; therefore, inactivating AMP-activated kinase (AMPK) and thus inhibiting insulin-stimulated glucose uptake. However, because etomoxir did not prevent the PPAR-induced decrease in lactate and pyruvate concentrations, it is unlikely that the rise in fatty acid oxidation caused glycolysis inhibition in adipocytes. Conversely, because adipocyte culture relies on glucose as energy substrate, the PPAR-induced decrease in glucose oxidation by allosteric enzymatic feedback controls (49). However, involvement of this cycle in our model is unlikely because we observed alterations in gene expression and because the etomoxir assay demonstrated that changes in glycolysis products were independent of fatty-acid cycle could be implied with β-oxidation limiting glucose oxidation by allosteric enzymatic feedback controls (49). However, involvement of this cycle in our model is unlikely because we observed alterations in gene expression and because the etomoxir assay demonstrated that changes in glycolysis products were independent of β-oxidation.

The metabolic alterations induced by PPAR contrast with the lack of PPARγ effect. This is puzzling given that PPARα and PPARγ target gene profiles are overlapping. There are, however, differences in induction of gene expression that may contribute to the selective effect of the

FIG. 4. PPARα agonist inhibits lipogenesis. Adipocytes were treated with GW7647 or vehicle. A, After 48 h treatment, palmitate incorporation into triglycerides was measured (n = 6); B and C, lipogenesis was assessed after the fate of [14C]glucose into fatty acids (B) and glycerol backbone of neutral lipids (C) (n = 13). *, P < 0.05.

FIG. 5. GW7647-induced metabolic changes are mediated by PPARα. A and B, Adipocytes were treated with 10 μM GW6471, a PPARα antagonist, and 1 μM PPAR agonist or vehicle. After 48 h treatment, lactate medium concentration was quantified (A), and palmitate oxidation was measured (B) (n = 6). *, P < 0.05. C and D, hMADS cells were differentiated into adipocytes and treated with agonists or vehicle for 48 h, whereas PPARα was knocked down with an siRNA (siPPARα) (n = 2). C, PPARα expression assessed by RT-qPCR. D, After treatment, palmitate oxidation was measured. Fold changes were calculated relative to DMSO/siRNA for green fluorescent protein (siGFP), and means ± SEM are plotted. Rosi, Rosiglitazone.
PPARα agonist. Regarding PPARα-induced up-regulation of fatty acid oxidation, CPT1B and HADHB showed a higher increase after GW7647 than rosiglitazone treatment. The data were confirmed at the protein level for HADHB. Moreover, CPT1 is allosterically inhibited by malonyl-CoA. Microarray data indicated that acetyl-CoA carboxylase-α (ACACA), which produces malonyl-CoA from acetyl-CoA, was significantly more repressed by PPARα than by PPARγ, whereas malonyl-CoA decarboxylase (MLYCD) was up-regulated by both agonists. Thus, the PPARα agonist may decrease malonyl-CoA concentration and further exacerbate lipid mitochondrial entry. However, given the marked difference in palmitate oxidation between the two agonists, other mechanisms may intervene.

The shift in glycolysis seemed to be linked to PPARα-induced coordinated down-regulation of many glycolysis genes, which was not seen with the PPARγ agonist. Because PDK4 has been shown as a gatekeeper responsible for the shift between glucose and fatty acid utilization, we investigated its regulation by PPAR agonists in human fat cells (50). Both agonists induced a robust reduction in PDK4 mRNA and protein levels. Therefore, regulation of PDK4 expression by GW7647 is unlikely to be responsible for glycolysis repression. It cannot, however, be ruled out that PDK4 enzyme activity is modified by posttranslational regulation. Finally, yet unidentified genes could be differentially regulated by PPARα and PPARγ. Recently, it was shown that CIDEB up-regulates lipid oxidation and decreases glucose oxidation in 3T3-L1 adipocytes, but in the present study, CIDEB was up-regulated by both agonists, ruling out its implication in this metabolic shift (51).

Altogether, these results highlight the role of PPARα in lipid and glucose homeostasis in human adipocytes. The relevance of this observation remains to be established in vivo. PPARα-induced lipid oxidation is of interest because it could promote fatty acid consumption in adipocytes and contribute to the control of body weight. In obese and insulin-resistant rats, fenofibrates reduce body weight gain and adiposity, which supports this hypothesis (52). We have previously shown that PPARα up-regulates glyceral phosphorylation, which when associated with an increase in lipolysis, could lead to fatty acid re-esterification into triglycerides and the creation of a futile cycle (22). Recently, it was shown that lactate inhibits lipolysis in adipocytes; thus, the PPARα-induced reduction in lactate concentration could contribute to enhanced lipolysis (53). Altogether, the combination of PPARα agonist and lipolysis enhancer may be envisioned as a tool to promote fatty acid consumption and thus diminish triglyceride stores.

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