The Transcriptional Coactivator Peroxisome Proliferator–Activated Receptor (PPAR)γ Coactivator-1α and the Nuclear Receptor PPARα Control the Expression of Glycerol Kinase and Metabolism Genes Independently of PPARγ Activation in Human White Adipocytes

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OBJECTIVE—The purpose of this work was to determine the pattern of genes regulated by peroxisome proliferator–activated receptor (PPAR) γ coactivator 1α (PGC-1α) in human adipocytes and the involvement of PPARα and PPARγ in PGC-1α transcriptional action.

RESEARCH DESIGN AND METHODS—Primary cultures of human adipocytes were transduced with a PGC-1α adenovirus and treated with PPARγ and PPARα agonists. Variation in gene expression was assessed using pangenomic microarrays and quantitative RT-PCR. To investigate glycerol kinase (GyK), a target of PGC-1α, we measured enzymatic activity and glycerol incorporation into triglycerides. In vivo studies were performed on wild-type and PPARα$^{-/-}$ mice. The GyK promoter was studied using chromatin immunoprecipitation and promoter reporter gene assays.

RESULTS—Among the large number of genes regulated by PGC-1α independently of PPARγ, new targets involved in metabolism included the gene encoding GyK. The induction of GyK by PGC-1α was observed at the levels of mRNA, enzymatic activity, and glycerol incorporation into triglycerides. PPARα was also upregulated by PGC-1α. Its activation led to an increase in GyK expression and activity. PPARα was shown to bind and activate the GyK promoter. Experiments in mice confirmed the role of PGC-1α and PPARα in the regulation of GyK in vivo.

CONCLUSIONS—This work uncovers novel pathways regulated by PGC-1α and reveals that PPARα controls gene expression in human white adipocytes. The induction of GyK by PGC-1α and PPARα may promote a futile cycle of triglyceride hydrolysis and fatty acid reesterification. ***Diabetes*** **56:2467–2475, 2007**

The accumulation of white adipose tissue (WAT) predisposes to the development of an array of metabolic disturbances leading to type 2 diabetes and cardiovascular disease. In a search for new therapies, it has been postulated that targeting molecular pathways that regulate thermogenesis may provide a plausible means of increasing energy expenditure (1). In that context, the opposite role of WAT specialized in energy storage in the form of triglycerides and brown adipose tissue (BAT) specialized in adaptive thermogenesis is of great interest. In humans, BAT depots and brown adipocytes are sparsely distributed in the body and are not thought to contribute to a significant part of adaptive thermogenesis (2,3). Conversion of human white adipocytes into fat cells with some properties of brown adipocytes is an attractive therapeutic strategy (4). Stimulation of lipolysis in WAT without the concomitant use of released fatty acids may be detrimental because fatty acids in excess will be deposited in other organs and may induce insulin resistance and cardiovascular complications. Instead, simultaneous activation of lipolysis and fatty acid utilization within white adipocytes could allow a decrease or a stabilization of fat mass without systemic side effects.

The transcriptional coactivator peroxisome proliferator–activated receptor γ (PPARγ) coactivator 1α (PGC-1α) was initially described as a metabolic regulator of adaptive thermogenesis in BAT (5). PGC-1α cooperates with the

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BAT, brown adipose tissue; GFP, green fluorescent protein; GSEA, Gene Set Enrichment Analysis; GyK, glycerol kinase; PANTHER, Protein Analysis Through Evolutionary Relationships; PGC-1α, peroxisome proliferator–activated receptor γ coactivator 1α; PPAR, peroxisome proliferator–activated receptor; PPRE, PPAR responsive element; ROS, reactive oxygen species; RXRα, retinoic acid X receptor-α; SLC25A4, solute carrier family 25 member 4; UCP, uncoupling protein; WAT, white adipose tissue.

heterodimer PPARγ/retinoic acid X receptor-α (RXRα) to stimulate the expression of the prototypical uncoupling protein (UCP)1, which allows a mitochondrial proton leak generating heat release. PGC-1α was later shown as essential for the control of mitochondriogenesis, fatty acid oxidation, and hepatic gluconeogenesis (6). These effects of PGC-1α are mediated by coactivation of other transcription factors (6,7). The coactivation of nuclear respiratory factor-1 and -2 and estrogen-related receptor-α was shown to be important for mitochondrial biogenesis. Interactions between PGC-1α and estrogen-related receptor-α or PPARα play a role in the regulation of fatty acid oxidation genes in heart and skeletal muscle (8,9).

Studies on human fat cells are a mandatory proof of concept to substantiate the strategy of white-to-brown fat-like adipocyte conversion. To determine whether it is possible to induce a metabolic shift from lipid storage toward fatty acid utilization, human subcutaneous white adipocytes, which express low levels of PGC-1α, have been modified to express human PGC-1α (10,11). We showed that PGC-1α coactivates the PPARγ/RXRα heterodimer to induce UCP1 expression. The present study was designed to get an exhaustive view in the transdifferentiation process through the determination of the profiles of genes regulated by PGC-1α in human adipocytes. We also wished to determine whether the changes in gene expression were attributable to interactions with PPARγ or other nuclear receptors. We found that PGC-1α regulates a large number of genes independently of the activation of PPARγ. Because an activation of PPARγ has been suggested to upregulate the expression of glycerol kinase (GyK) and thereby promote a futile cycle of triglyceride breakdown and resynthesis (12), we investigated the mechanisms controlling GyK, one of the PGC-1α targets, in human white adipocytes. The expression of GyK was not stimulated by a PPARγ agonist. However, GyK expression and biological effect was induced by PPARα. Our data suggest a model in which PGC-1α induces PPARα, which appears as one of the regulators of GyK expression.

RESEARCH DESIGN AND METHODS

Differentiation of human preadipocytes, treatment with agonists, and adenovirus infection. Subcutaneous abdominal WAT was obtained from female subjects undergoing plastic surgery in agreement with French laws on biomedical research. Human adipocytes in primary culture were differentiated as described previously (11,13). Adenoviruses produced at the Gene Therapy laboratory of Nantes contained, in tandem, the green fluorescent protein (*GFP*) gene and the PGC-1α cDNA downstream of separate cytomegalovirus promoters. An adenovirus containing only the *GFP* gene was used as control. Cells were infected at day 13 with the PGC-1α or control adenovirus at a multiplicity of infection of 200 for 6 h unless otherwise indicated. The next day, cells were treated as indicated in text and figure legends with the following drugs at 1 μmol/l: rosiglitazone (BRL49653; Smith Kline and French), GW7647 (Sigma), and 9-*cis*-retinoic acid (Sigma). Cells were harvested after 48 h of treatment for mRNA assays and 96 h of treatment for protein, enzymatic activity, and glycerol incorporation assays unless otherwise indicated. PPAR agonists and retinoic acid were prepared in DMSO and then diluted in medium. Vehicle (DMSO at 0.1%) was added to control cells.

Human adipose tissue explants. Cultures of human subcutaneous adipose tissue explants were performed as described previously (14). After 24 h of treatment with rosiglitazone (1 μmol/l) or vehicle (DMSO), tissue pieces were collected for mRNA analysis.

Microarray data analysis. We analyzed the transcriptional program of human adipocytes overexpressing PGC-1α and/or treated with rosiglitazone plus 9-*cis*-retinoic acid. Microarrays exhibiting 41,805 cDNA spots representing 41,126 IMAGE clones and 24,474 Unique Putative Genes were from Stanford University (15). Between 30,313 and 31,453 spots were recovered from each array slide. To assess differentially expressed genes, we used the Varmixt method that allows a control of the heterogeneity of variance (16). This procedure yielded 1,746 clones with q value <0.05 representing 1,622 genes that were common to all of the three treatments when compared with control cells. Hierarchical agglomerative cluster analysis was performed using the Euclidian distance as a measure of similarity and the Ward's method as the agglomeration criteria. The dendrogram obtained was cut in six groups. To analyze the function of the differentially expressed genes, we used the Protein Analysis Through Evolutionary Relationships (PANTHER) system database with focus on the biological process ontology. To test for the enrichment of sets of genes in expression data, we used the statistical method Gene Set Enrichment Analysis (GSEA) together with the functional database dedicated to microarrays from Stanford.

Quantitative RT-PCR analysis. Reverse transcription and real-time quantitative PCR (Applied Biosystems) was performed using SYBR green chemistry (Supplementary Table 1, which is available in the online appendix at http://dx.doi.org/10.2337/db06-1465). Some mRNAs were quantified using pre-made gene expression assays (Applied Biosystems). 18S ribosomal RNA was used as control to normalize gene expression.

Protein, enzymatic, and metabolic analyses. For PGC-1α Western blot analysis, total proteins from human adipocytes were separated on SDS-PAGE, transferred onto nitrocellulose membrane, and probed with a polyclonal anti-human PGC-1α (H-300) antibody (Santa Cruz Biotechnology). To determine metallothionein quantities in adipocytes, cytoplasmic proteins from human adipocytes were prepared in Tris-HCl (20 mmol/l, pH 8) buffer. Metallothionein measurement was realized by a radioimmunoassay (17). GyK activity and glycerol incorporation into triglycerides were assessed according to Noel et al. (18) and Guan et al. (12), respectively.

Transfection and luciferase assays. Transfection experiments were performed in 3T3-F442A preadipocytes. Transient transfections were performed by LipofectAMINE 2000 reagent (Invitrogen). A GyK promoter fragment from −2007 to +44 was amplified by PCR from human genomic DNA (Roche Diagnostics) using Platinum high fidelity DNA polymerase (Invitrogen). The PCR product was cloned into the pGL3 basic (Promega). Point mutations of PPAR responsive element (PPRE) present on the GyK promoter were achieved by site-directed mutagenesis (Stratagene). Three copies of the PPRE of the human GyK promoter (AAAGTATGTGAAAGTTCAAACGT) were cloned in front of the thymidine kinase promoter in the pGL3 basic plasmid. The reporter plasmid was cotransfected with expression vectors for PGC-1α, PPARα, PPARγ, and RXRα. A β-galactosidase–expressing plasmid was used for transfection normalization. The experiments were performed in triplicates. Forty-eight hours after the transfection, luciferase activities were analyzed using the Luciferase Reporter Assay System (Promega).

Chromatin immunoprecipitation experiments. Chromatin immunoprecipitation (ChIP) experiments were performed according to Annicotte et al. (19). Immunoprecipitation was carried out using IgG antibodies, affinity-purified anti-PPARα polyclonal antibody (Santa Cruz), or affinity-purified anti-acetyl histone H4 (Cell Signaling). Purified DNA was amplified using GyK promoter–specific primers and quantified by real-time PCR using SYBR Green chemistry (Supplementary Table 1). Primers targeting a genomic region localized in the human UCP2 gene were used to control for nonspecific binding.

Animal studies. Studies with mice followed the INSERM and Louis Bugnard Institute Animal Care Facility guidelines. Wild-type or PPARα-null mice were treated by daily gavage with Wy14643 (30 $mg \cdot kg^{-1} \cdot day^{-1}$) (Sigma) or vehicle (3% carboxymethylcellulose) for 7 days. In vivo adenovirus injection (10^{10} infectious particles) were performed in epididymal fat pads of male B6D2/F1 mice. A fat pad was injected with PGC-1α adenovirus and the contralateral fat pad with GFP adenovirus. Tissues were collected after 3 days. For cold exposure experiments, animals were maintained at 4°C for 48 h.

Statistical methods. Data are expressed as means ± SE. The data were compared using the nonparametric Wilcoxon's test.

Microarray dataset. The microarray data for this study are fully accessible at Gene Expression Omnibus with accession number GSE5184.

RESULTS

Characterization of genes regulated by the transcriptional coactivator PGC-1α and by agonists for the nuclear receptors PPARγ and RXRα in human adipocytes. To gain insight in the respective action of PGC-1α and PPARγ/RXRα in human fat cells, microarray experiments were performed on primary cultures of human subcutaneous differentiated adipocytes transduced with an adenovirus encoding the human form of PGC-1α, treated with rosiglitazone and retinoic acid, or received a combination of both treatments. Only quiescent mature adipocytes were transduced by the adenovirus. This

FIG. 1. Upregulation of PGC-1α target genes in human white adipocytes. Gene expression was determined in human differentiated adipocytes transduced with an adenovirus expressing PGC-1α or treated with rosiglitazone (Rosi) and retinoic acid (RA). *A*: PGC-1α and PGC-1β mRNA levels in human white adipocytes expressing PGC-1α ($n = 4$; $^*P < 0.05$). *B*: Western blot of PGC-1α in cellular extracts of human white adipocytes expressing PGC-1α. *C*: Isocitrate dehydrogenase 3, α-subunit (IDH3A) mRNA. *D*: SLC25A4 (adenine nucleotide translocator 1) mRNA. *E*: Parvalbumin (PVALB) mRNA. *F*: p53-induced gene 3 (PIG3) mRNA. *G*: MT1F mRNA ($n \geq 4$; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). *H*: Metallothionein (MT) protein ($n = 6$; $^{***}P < 0.001$). Data are expressed as fold change relative to control adipocytes.

method permits to avoid the effect of continuous PGC-1α expression during adipogenesis. Transduction with the PGC-1α adenovirus led to an increase in PGC-1α mRNA and protein expression, whereas a 30% decrease in PGC-1β mRNA level was observed (Fig. 1*A* and *B*). Using a novel statistical method for the differential analysis of gene expression (16) and cluster analysis, we defined six mutually exclusive groups of genes (Supplementary Fig. 1; Supplementary Table 2). PGC-1α alone induced the expression of 125 genes. Very few genes (11) were downregulated in PGC-1α overexpressing adipocytes. These data show that PGC-1α acts almost exclusively as a coactivator. Rosiglitazone and retinoic acid up- and downregulated 245 and 331 genes, respectively.

To determine which biological processes were affected by the treatments, we used the PANTHER ontological classification system. As expected, the treatment with

TABLE 1
Genes upregulated by rosiglitazone and retinoic acid or by PGC-1α classified according to biological processes by the PANTHER software

Treatment	Biological process	Genes			*P* value
		In the pathway	Expected	Regulated	
Rosiglitazone and retinoic acid	Lipid, fatty acid, and steroid metabolism	525	10	24	2×10^{-3}
	Fatty acid metabolism	118	2	11	2×10^{-3}
	Amino acid activation	37	1	6	1×10^{-2}
	Protein metabolism and activation	1,794	33	52	2×10^{-2}
	Protein folding	116	2	9	5×10^{-2}
	Protein targeting and mobilization	149	3	9	6×10^{-2}
PGC-1α	Electron transport	166	2	14	2×10^{-8}
	Oxidative phosphorylation	42	1	8	1×10^{-6}
	Tricarboxylic acid pathway	22	1	5	3×10^{-4}
	Carbohydrate metabolism	381	4	12	7×10^{-3}

Data are *n*. Using a binomial statistical tool, the number of regulated genes is compared with the number of genes expected by chance for each pathway. Only pathways statistically overrepresented ($P < 0.05$) are indicated.

TABLE 2
Confirmation of microarray data analysis by quantitative RT-PCR

Treatment	Gene symbol	Gene name	Gene Ontology annotation (accession no.)	Microarray	Quantitative RT-PCR
Rosiglitazone and retinoic acid	ANGPTL4	Angiopoietin-like 4, FIAF	Enzyme inhibitor activity (0004857)	6.07	4.61*
	LBP	Lipopolysaccharide binding protein	Lipid binding (0008289)	4.73	3.72†
	LPL	Lipoprotein lipase	Lipoprotein lipase activity (0004465)	2.48	1.59†
	NR1H3	Nuclear receptor subfamily 1, group H, member 3, LXRα	Transcription (0006350)	2.26	3.32†
	PCK1	Phosphoenolpyruvate carboxykinase 1 (soluble)	PEPCK (GTP) activity (0004613)	2.20	3.23‡
	FABP4	Fatty acid binding protein 4, adipocytes, ap2	Fatty acid binding (0005504)	2.19	2.85†
	MMP9	Matrix metallopeptidase 9	Collagenase activity (0008133)	0.77	0.53†
	AACS	Acetoacetyl-CoA synthetase	CoA-ligase activity (0016405)	0.40	0.43†
PGC-1a	PVALB	Parvalbumin	Calcium ion binding protein (0005509)	7.35	484‡
	GOT1	Glutamic-oxaloacetic transaminase 1	Aspartate transaminase activity (0004069)	5.10	7.23‡
	MT1F	Metallothionein 1F	Metal ion binding (0046872)	4.89	80*
	SLC25A4	Solute carrier family 25, member 4, ANT1	Adenine transporter activity (0015207)	4.65	7.23‡
	MT1X	Metallothionein 1X	Metal ion binding (0046872)	4.25	14*
	IDH3A	Isocitrate dehydrogenase 3 (NAD+)-α	Oxidoreductase activity (0016491)	3.28	8.69†
	PPARα	Peroxisome proliferator –activated receptor-α	Ligand-dependent nuclear receptor activity (0004879)	1.89	1.56†
	CYCS	Cytochrome c, somatic	Electron transporter (0045155)	1.84	2.67†
	PIG3	P53-induced gene 3	Oxidoreductase activity (0016491)	1.64	3.11‡
	GYK	Glycerol kinase	Glycerol kinase activity (0004370)	1.52	4.80‡
	MGST2	Microsomal glutathione S-transferase 2	Glutathione transferase activity (0004364)	1.45	1.95†

Data are fold change. Normalization was performed with 18S mRNA. *$P < 0.001$, †$P < 0.05$, ‡$P < 0.01$.

rosiglitazone and retinoic acid induced changes in the expression of genes involved in lipid metabolism (Table 1). Less expected, a significant number of genes involved in protein metabolism were upregulated. Regarding biological processes induced by PGC-1α overexpression, several families of genes expressed in mitochondria were identified. The regulation of sets of mitochondrial genes by PANTHER was confirmed using GSEA (Supplementary Table 3; Supplementary Fig. 2). These data reveal that a large number of genes involved in mitochondrial energy metabolism are regulated by PGC-1α independently of PPARγ/RXRα activation.

We confirmed microarray data by determination of mRNA levels using quantitative RT-PCR (Table 2; Fig. 1*C–H*). An excellent concordance was observed between the two techniques for gene targets of PPARγ/RXRα and PGC-1α. Known target genes of PGC-1α involved in mitochondrial energy metabolism, such as isocitrate dehydrogenase 3 and solute carrier family 25 member 4 (SLC25A4) (adenine nucleotide translocase 1), were upregulated in PGC-1α–overexpressing cells but not in cells treated only with rosiglitazone and retinoic acid (Fig. 1*C* and *D*). The regulation of new target genes, such as parvalbumin, a cytosolic calcium ion binding protein involved in muscle relaxation, p53-induced gene 3, which codes for an enzyme involved in cellular responses to oxidative stress, and microsomal glutathione *S*-transferase 2, an enzyme involved in leukotriene formation, was also confirmed (Fig. 1*E* and *F*; Table 2). Several metallothionein isoforms (MT1B, -F, -G, -H, -K, -L, and -X and MT2A) were induced by PGC-1α overexpression in microarray experiments (Supplementary Table 2). Metallothioneins are low–molecular weight cysteine-rich proteins that have the ability to bind and sequestrate heavy metal ions. In humans, metallothionein genes are clustered within the q13 region on chromosome 16 and include at least 11 MT1 members. MT1F and MT1X mRNA upregulation was confirmed by quantitative RT-PCR (Fig. 1*G*; Table 2). Moreover, total metallothionein protein level was shown to be induced by PGC-1α expression (Fig. 1*H*).

GyK is regulated by PGC-1α but not by rosiglitazone. GyK gene was among the genes regulated by PGC-1α but not by rosiglitazone and retinoic acid. These data were surprising because previous studies have shown an induction of GyK by rosiglitazone in murine 3T3-L1 adipocytes (12). Therefore, we analyzed GyK expression and activity in several human models. The induction by PGC-1α and lack of effect of rosiglitazone was confirmed by quantitative RT-PCR in human differentiated adipocytes (Fig. 2*A*). The PPARγ agonist was also devoid of effect in human adipose tissue explants composed of mature adipocytes (Fig. 2*B*). Furthermore, GyK activity was increased only in human adipocytes overexpressing PGC-1α (Fig. 2*C*). Induction of PGC-1α by thiazolidinediones and recruitment of the coactivator to the GyK gene has been proposed as the model explaining GyK upregulation by rosiglitazone in 3T3-L1 adipocytes (20). PGC-1α mRNA expression was not regulated by rosiglitazone either in differentiated human adipocytes or in explants of adipose tissue (Fig. 2*D* and *E*).

An induction of GyK activity, which is normally very low in white adipocytes, suggests a role in the production of glycerol-3-phosphate, which allows the creation of a futile cycle of triglyceride hydrolysis and resynthesis (12). However, other enzymes may participate in the production of glycerol-3-phosphate. The cytoplasmic and mitochondrial

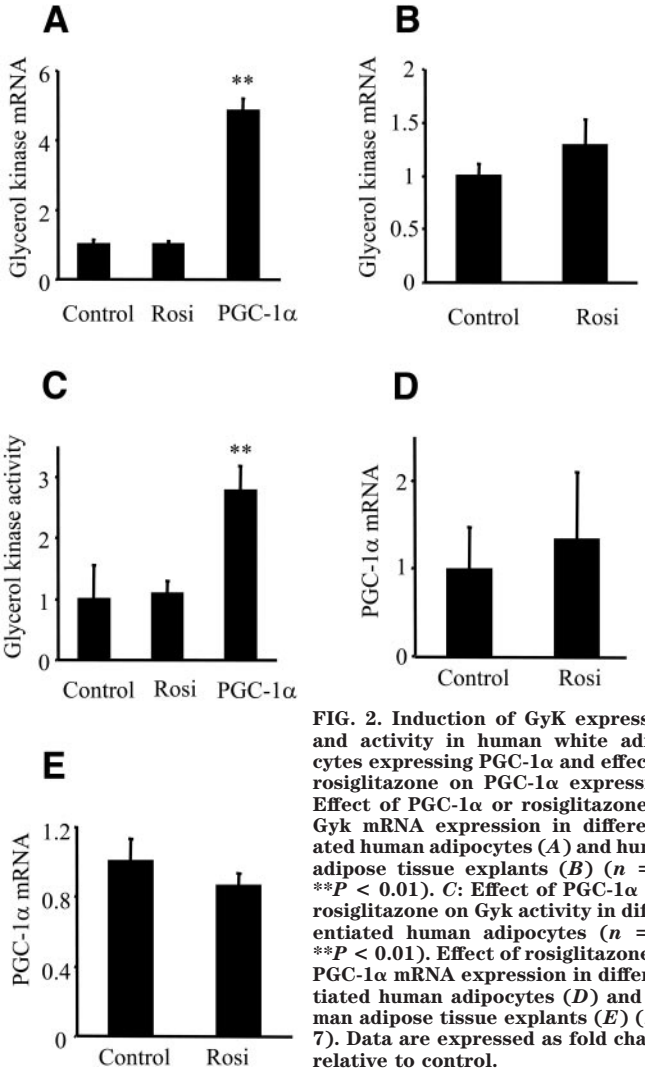

FIG. 2. Induction of GyK expression and activity in human white adipocytes expressing PGC-1α and effect of rosiglitazone on PGC-1α expression. Effect of PGC-1α or rosiglitazone on Gyk mRNA expression in differentiated human adipocytes (*A*) and human adipose tissue explants (*B*) ($n = 7$; $^{**}P < 0.01$). *C*: Effect of PGC-1α and rosiglitazone on Gyk activity in differentiated human adipocytes ($n = 5$; $^{**}P < 0.01$). Effect of rosiglitazone on PGC-1α mRNA expression in differentiated human adipocytes (*D*) and human adipose tissue explants (*E*) ($n = 7$). Data are expressed as fold change relative to control.

forms of glycerol-3-phosphate dehydrogenase and PEPCK were not regulated by PGC-1α (Supplementary Fig. 3).

PPARα participates in GyK regulation. PPARα was one of PGC-1α targets (Fig. 3*A*). To determine an implication of PPARα in GyK gene regulation, we treated human adipocytes overexpressing or not PGC-1α with a selective agonist of PPARα, GW7647. Treatment by GW7647, adenofection of PGC-1α, and the association of both induced GyK gene expression and activity (Fig. 3*B* and *C*). The induction of GyK was accompanied by an increase of glycerol incorporation into triglycerides after PPARα activation and PGC-1α overexpression (Fig. 3*D*). The concomitant time course of PPARα and GyK mRNA induction in PGC-1α–expressing adipocytes was coherent with a role of PPARα in the regulation of GyK expression (Fig. 3*E* and *F*).

Induction of PGC-1α in white fat cells is associated with an upregulation of PPARα and GyK expression. We then investigated whether overexpression of PGC-1α could induce GyK and PPARα expression in vivo. The PGC-1α or GFP adenoviruses were injected in epididymal fat pads of mice. This fat pad was selected because it contains very few brown fat cells. The increase in PGC-1α mRNA expression was accompanied by a comparable increase of GyK and PPARα mRNA levels (Fig. 4*A*). A twofold induction of PGC-1α expression was observed in epididymal white fat pad exposed to cold for 48 h (Fig.

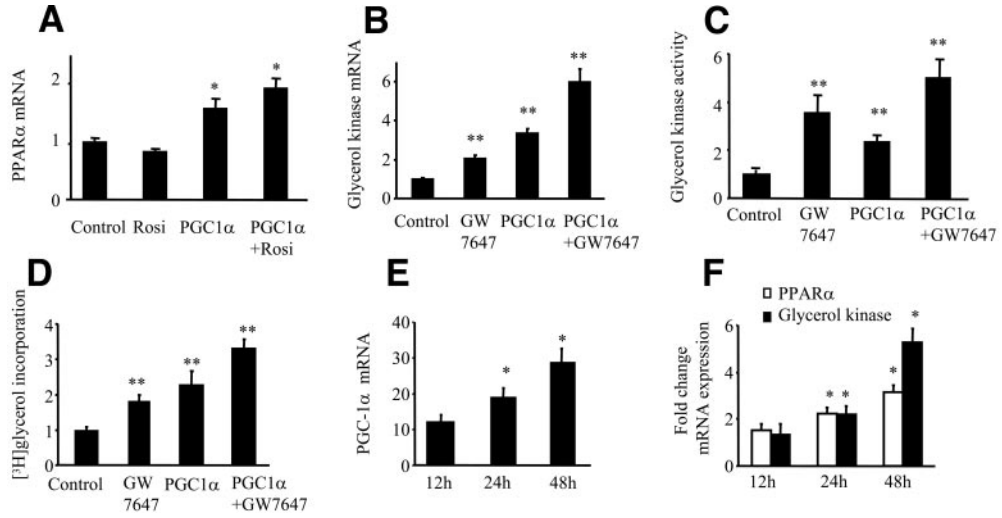

FIG. 3. PPARα-mediated induction of GyK in human white adipocytes. *A*: Effect of PGC-1α and rosiglitazone on PPARα mRNA ($n = 11$; *$P < 0.05$). Effect of PGC-1α and GW7647, a PPARα agonist, on GyK mRNA (*B*) ($n = 10$; **$P < 0.01$), GyK activity (*C*) ($n = 6$; **$P < 0.01$), and [^{3}H]glycerol incorporation into triglycerides normalized by protein quantity (*D*) ($n = 3$; **$P < 0.01$). Kinetic analysis of adipocytes transduced with PGC-1α adenovirus on PGC-1α mRNA (*E*) and PPARα and Gyk mRNA (*F*). Data are expressed as fold change relative to control adipocytes.

4*B*). The increase in PGC-1α mRNA expression was associated with a comparable induction of PPARα and GyK gene expression. We investigated the involvement of PPARα in the regulation of GyK expression in WAT of PPARα-null mice. Wild-type and PPARα$^{-/-}$ mice were treated with the PPARα agonist (WY 14643). An induction of GyK mRNA was observed in control mice treated with WY14643 but not in PPARα$^{-/-}$ mice (Fig. 4*C*). As previously reported, the WAT expression of GyK was higher in PPARα$^{-/-}$ mice than in wild-type mice (21).

PPARα activates GyK gene transcription. A functional PPRE has been identified in the murine GyK promoter (20). This PPRE is perfectly conserved in the human promoter (Fig. 5*A*). To investigate the role of PPARα in the transcription of the GyK gene, we transfected a human GyK promoter construct linked to the luciferase gene with expression vectors for PPARα, RXRα, and PGC-1α into 3T3-F442A cells. Cells were also treated with the PPARα agonist, GW7647. PGC-1α and the heterodimer PPARα/RXRα induced an increase in luciferase activity (Fig. 5*B*). A slightly greater induction was observed in cells expressing both PGC-1α and PPARα/RXRα and treated with GW7647. Mutation of the PPRE abolished induction by PPARα/RXRα in the presence of GW7647. An induction was observed with PGC-1α, indicating possible interaction of the coactivator with other transcription factors (Fig. 5*C*). To get a direct demonstration of the involvement of the GyK PPRE in PPARα-mediated transactivation and to compare PPARα and PPARγ effects, PPRE was cloned upstream of a minimal thymidine kinase promoter. Transfection into 3T3-F442A cells revealed a robust induction by PPARα/RXRα, whereas PPARγ/RXRα showed poor transactivation capacity (Fig. 5*D*). Moreover, PGC-1α had little effect on luciferase activity, suggesting that PPARα is able to transactivate the human GyK promoter independently of PGC-1α. ChIP experiments were performed to investigate whether PPARα binds the Gyk PPRE (Fig. 5*E*). Recruitment of endogenous PPARα to the GyK PPRE was observed in human adipocytes overexpressing PGC-1α. The concomitant presence of acetylated histone H4 in the GyK PPRE suggests that the promoter is transcriptionally active when PPARα is bound. Binding of PPARα on an unrelated genomic region was not enhanced in PGC-1α–expressing adipocytes.

DISCUSSION

Alterations in fatty acid metabolism and mitochondrial dysfunction are thought to be critical in the genesis of insulin resistance (22). The transcriptional coactivator

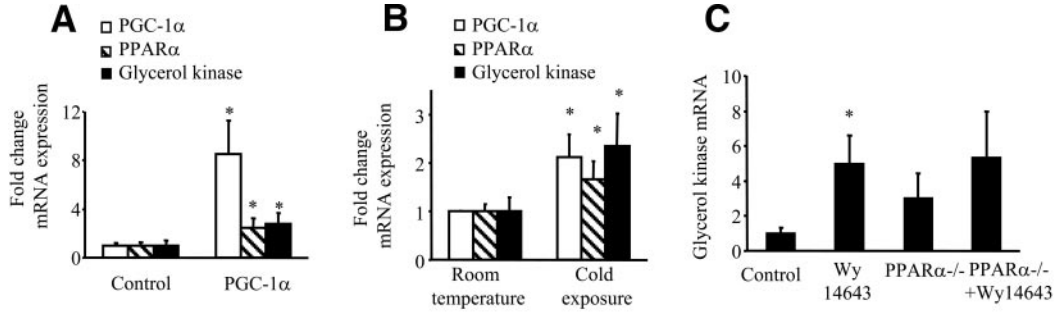

FIG. 4. In vivo regulation of GyK gene expression through PGC-1α– and PPARα-dependent mechanisms in mouse WAT. *A*: PGC-1α, PPARα, and GyK mRNA levels in epididymal fat pads injected with PGC-1α adenovirus ($n = 4$; *$P < 0.05$). *B*: PGC-1α, PPARα, and GyK mRNA levels in epididymal WAT of mice exposed to cold ($n = 6$; *$P < 0.05$). *C*: GyK mRNA levels in epididymal fat pads of PPARα-null and control mice treated daily by gavage with a PPARα agonist, Wy14643 ($n = 5$; *$P < 0.05$). Data are expressed as fold change relative to control fat.

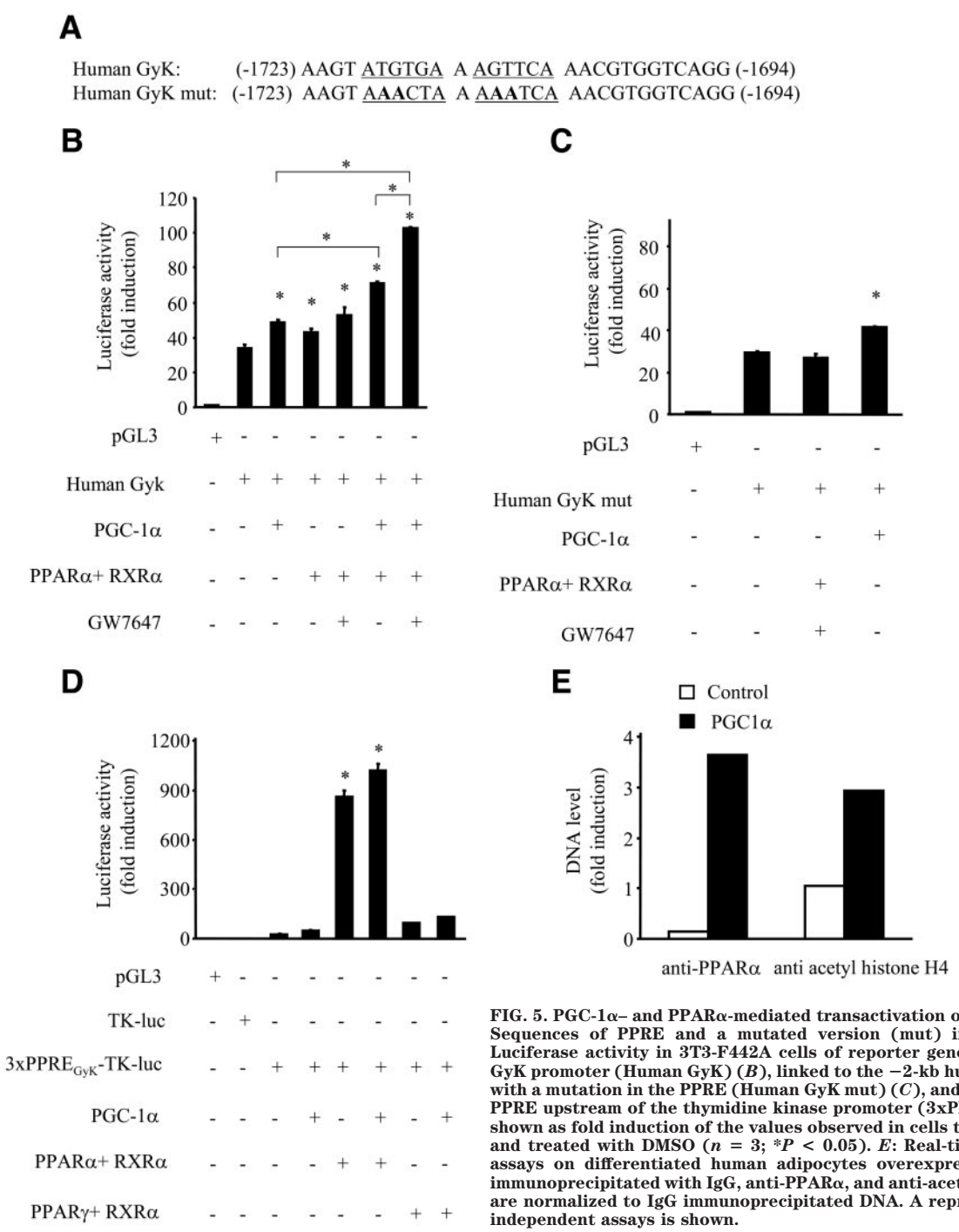

FIG. 5. PGC-1α– and PPARα-mediated transactivation of the human GyK promoter. *A*: Sequences of PPRE and a mutated version (mut) in the human GyK promoter. Luciferase activity in 3T3-F442A cells of reporter genes linked to the −2-kb human GyK promoter (Human GyK) (*B*), linked to the −2-kb human glycerol kinase promoter with a mutation in the PPRE (Human GyK mut) (*C*), and containing three copies of the PPRE upstream of the thymidine kinase promoter (3xPPRE$_{Gyk}$-TK-Luc) (*D*). Data are shown as fold induction of the values observed in cells transfected with empty vectors and treated with DMSO ($n = 3$; *$P < 0.05$). *E*: Real-time PCR quantitation of ChIP assays on differentiated human adipocytes overexpressing PGC-1α. Samples were immunoprecipitated with IgG, anti-PPARα, and anti-acetyl histone H4 antibodies. Data are normalized to IgG immunoprecipitated DNA. A representative experiment of five independent assays is shown.

PGC-1α is pivotal in these pathways because it controls genes involved in fatty acid oxidation and mitochondrial respiratory chain (6). In this report, we performed an exhaustive analysis of PGC-1α and PPARγ/RXRα target genes in human white fat cells. We identified genes belonging to various pathways that were regulated independently by PGC-1α or PPARγ/RXRα. Among these genes was GyK, a key enzyme in fatty acid esterification. A model is proposed to recapitulate how PGC-1α and PPARα trigger GyK expression and metabolic function (Fig. 6). In human fat cells, PPARα is induced by PGC-1α, and its activation leads to an increase in GyK expression and glycerol incorporation into triglycerides. In vivo data in the mouse support the role of PPARα in the regulation of GyK. Studies of the human GyK promoter show that the nuclear receptor acts through a PPRE located at −1.7 kb. Interestingly, GyK expression data in human adipocytes and promoter studies reveal that PGC-1α is dispensable for PPARα-mediated transactivation. Moreover, in vitro and in vivo data suggest that other transcription factors are involved in PGC-1α–mediated upregulation of GyK.

Gene clustering analysis and classification according to functional annotations show that numerous pathways are regulated by PGC-1α independently of the activation of the PPARγ/RXRα heterodimer. Induction of PGC-1α expression in human adipocytes induces mitochondrial genes, as observed in skeletal muscles, hepatocytes, and 3T3-F442A preadipose cells (5,6,23). Analysis of biological processes shows that enzymes of the citric acid cycle and proteins of the respiratory chain are upregulated in human adipo-

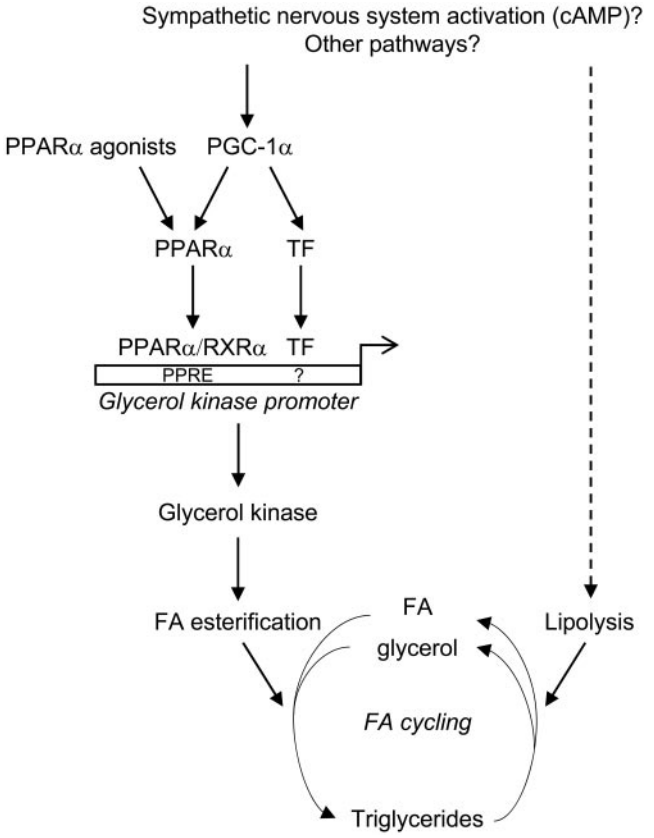

FIG. 6. Model of activation of fatty acid (FA) recycling through induction of glycerol kinase by PGC-1α, PPARα, and unknown transcription factors (TF).

cytes. Among the gene sets selected by GSEA analysis, we found genes involved in reactive oxygen species (ROS) metabolism. PGC-1α could protect cells against an increase in ROS production resulting from its capacity to induce mitochondrial gene expression and thus a higher mitochondrial activity. Several isoforms of metallothionein were upregulated, and metallothionein protein quantity was increased by PGC-1α. MT1 is induced in BAT by cold and catecholamines, two conditions that induce PGC-1α (24). Accordingly, we found that MT1 gene expression was increased during cold exposure in mouse WAT (data not shown). Besides a role in metal metabolism, metallothionein may protect cells against oxidative stress (25). Because ROS have been recently proposed to play a causal role in multiple forms of insulin resistance (26), PGC-1α could contribute to the maintenance of insulin sensitivity through induction of protective genes, such as metallothionein. Induction of metallothionein genes may also be beneficial for the control of energy balance as MT1- and MT2-null mice develop obesity (27).

The predominant pathway for fatty acid esterification to form triglycerides in adipocytes starts with glycerol-3-phosphate. GyK catalyzes direct conversion of glycerol to glycerol-3-phosphate. By comparison with BAT, GyK activity in human WAT is low (28). In human white fat cells, we showed that PGC-1α strongly induces GyK gene expression and activity. The increase in glycerol incorporation into triglycerides after GyK upregulation allows a direct recycling of glycerol resulting from a complete hydrolysis of triglycerides (12). The existence of a triglyceride/fatty acid cycle has been demonstrated in physiological and pathological situations in humans (29). Therefore, in human white adipocytes expressing PGC-1α, a futile cycle induced by Gyk and the stimulation of fat oxidation could limit the release of fatty acids by WAT and participate in the beneficial modification of metabolism (Fig. 6) (4,11).

Unlike what has been reported in mouse adipocytes (12), we did not find any stimulation of GyK expression by rosiglitazone in human primary adipocytes and adipose tissue explants. The lack of induction of GyK mRNA by rosiglitazone has been confirmed in human adipose tissue explants (30). No change in Gyk mRNA expression has been observed in WAT of type 2 diabetic patients treated with thiazolidinediones (31,32). Fasting glycerol output from WAT was not decreased during a rosiglitazone treatment as would be expected if Gyk activity was upregulated (31). Moreover, elegant in vivo experiments using heavy water showed that, under rosiglitazone treatment, GyK is unlikely to contribute to triglyceride synthesis in adipose tissue (33). The lack of induction in human fat cells is probably related to the absence of PGC-1α upregulation because this mechanism has been proposed as essential for the induction of GyK by rosiglitazone in 3T3-L1 adipocytes (20). Next, we searched to identify which nuclear receptor cooperates with PGC-1α to regulate GyK expression. We found that PGC-1α upregulates PPARα expression in human adipocytes. Moreover, a selective agonist of PPARα increased GyK expression and activity in our model. Furthermore, promoter-reporter gene assays showed that the nuclear receptor activates human GyK transcription, and ChIP experiments revealed that PPARα was directly recruited to the GyK promoter in PGC-1α–expressing human adipocytes. Treatment of human white fat cells with forskolin, an adenylylase activator, upregulates PGC-1α and PPARα mRNA expression (34). We observed that forskolin increased GyK mRNA level in human adipocytes (data not shown). In situations of chronic activation of white fat cells by catecholamines, high intracellular cAMP levels could lead to a stimulation of lipolysis and an induction of PGC-1α, PPARα, and GyK expression promoting fatty acid esterification (Fig. 6).

The data raise the interesting possibility of a role for PPARα in regulating white fat metabolism. PPARα deficiency in mice results in disturbances of triglyceride and cholesterol metabolism in WAT with alterations in lipogenic gene expression (35). Besides the well-established systemic effect of PPARα agonists on energy expenditure and fat oxidation, it has recently been suggested that activation of the nuclear receptor in WAT could prevent adipocyte hypertrophy (36). In line with this concept, PPARα is essential for the lipopenic effect of hyperleptinemia on WAT (37). Furthermore, PPARα mediates the action of a β_3-adrenergic receptor agonist on WAT gene expression and remodeling (21). These data on mouse WAT shall however be interpreted with caution because important species differences exist between mouse and human white fat cell metabolism (38). However, our data show the direct involvement of PPARα in the control of human adipocyte gene expression. This study paves the way for future work aiming at characterizing the biological pathways modulated by PPARα agonists in human fat.

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