Adipocyte-specific Gene Expression and Adipogenic Steatosis in the Mouse Liver Due to Peroxisome Proliferator-activated Receptor γ1 (PPARY1) Overexpression*

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Peroxisome proliferator-activated receptor (PPAR) isoforms, α and γ, function as important coregulators of energy (lipid) homeostasis. PPARα regulates fatty acid oxidation primarily in liver and to a lesser extent in adipose tissue, whereas PPARγ serves as a key regulator of adipocyte differentiation and lipid storage. Of the two PPARγ isoforms, PPARγ1 and PPARγ2 generated by alternative splicing, PPARγ1 isoform is expressed in liver and other tissues, whereas PPARγ2 isoform is expressed exclusively in adipose tissue where it regulates adipogenesis and lipogenesis. Since the function of PPARγ1 in liver is not clear, we have, in this study, investigated the biological impact of overexpression of PPARγ1 in mouse liver. Adenovirus-PPARγ1 injected into the tail vein induced hepatic steatosis in PPARα−/− mice. Northern blotting and gene expression profiling results showed that adipocyte-specific genes and lipogenesis-related genes are highly induced in PPARα−/− livers with PPARγ1 overexpression. These include adipin, adiponectin, aP2, caveolin-1, fasting-induced adipose factor, fat-specific gene 27 (FSP27), CD36, Δ3 desaturase, and malic enzyme among others, implying adipogenic transformation of hepatocytes. Of interest is that hepatic steatosis per se, induced either by feeding a diet deficient in choline or developing in fasted PPARα−/− mice, failed to induce the expression of these PPARγ-regulated adipogenesis-related genes in steatotic liver. These results suggest that a high level of PPARγ in mouse liver is sufficient for the induction of adipogenic transformation of hepatocytes with adipose tissue-specific gene expression and lipid accumulation. We conclude that excess PPARγ activity can lead to the development of a novel type of adipogenic hepatic steatosis.

of adipogenesis (1–4). The PPAR subfamily consists of three isoforms, namely PPARα, PPARγ, and PPARβ/δ, and like all other nuclear receptors, PPARs possess a highly conserved DNA-binding domain that recognizes peroxisome proliferator response elements (PPREs) in the promoter regions of target genes (5–9). After ligand binding PPARs heterodimerize with retinoid-X-receptors (RXR) and PPAR-RXR heterodimers bind to PPRE to initiate the transcriptional regulation of target genes, in particular those involved in lipid homeostasis (5–10). The three PPAR isoforms are products of separate genes, and they exhibit distinct patterns of tissue distribution (7). PPARγ is expressed at a relatively high concentration in liver, plays a central role in regulating enzymes involved in the oxidation of fatty acids, and is essential for the pleiotropic responses induced in liver by structurally diverse chemicals known as peroxisome proliferators (8–10). PPARγ is present in two isoforms, PPARγ1 and PPARγ2, resulting from alternate promoter usage (1, 8, 9). PPARγ2 contains an additional 30 amino acids at the N-terminal end relative to PPARγ1. PPARγ2 expression is limited exclusively to adipose tissue where it play a key role in adipogenesis (1, 2). On the other hand, PPAR γ1 is expressed at relatively low levels in many tissues including liver, but the function of this isoform in non-adipose tissue locations is not well delineated (1, 9, 13). Forced expression of PPARγ2 or PPARγ1 can initiate the differentiation of fibroblasts to adipocytes, and in the process the fibroblasts express adipocyte-specific genes and accumulate lipid (2, 14). Although there is some suggestion that PPARγ ligands may induce adipocyte-specific gene expression in certain tumor cells, it is uncertain as to whether PPARγ1 or PPARγ2 expression in vivo, in non-fibroblast mesenchymal cells, or in epithelial tissues can lead to adipocyte-specific gene expression and adipogenesis (15).

It is well known that CCAAT/enhancer binding family of transcription factors C/EBPs, β, and δ have been shown to play an important role in adipogenesis in that high expression of each member of this family will direct fibroblasts to differentiate into adipocytes, and this conversion is mediated through down-stream regulator PPARγ and PPAR coactivator PPAR/TRAP220/DRIP205 (4, 16–19). C/EBPδ and PPARβ are expressed in liver, but the significance of this expression in terms of adipogenesis or lipid accumulation in liver cells remains unclear. The inability of C/EBP and PPARβ to induce adipogenesis in normal hepatocytes may be due to the fact that down-stream regulator PPARγ (PPARγ1 in liver) may be rate-limiting in hepatocytes (19–21). In this study, we used an adenoviral gene delivery system to overexpress PPARγ1 in mouse liver to determine whether this would trigger the expression of adipocyte-specific genes and lipid accumulation (steatosis) in liver cells. To avoid the confounding effect of PPARαs, we used PPARα−/− mice (22), and found that overex-
pression of PPARγ1 in these livers leads to adipocyte-specific gene expression and lipid accumulation. We also demonstrate that fatty liver induced by starvation or that developing after feeding a diet deficient in choline is not associated with the induction of genes associated with adipogenesis unlike that accompanying PPARγ1 overexpression in liver. These results strongly suggest that the low level of PPARγ1 appears to prevent liver cells from becoming adipocytes despite the prominence of C/EBPα gene expression in these cells and that overexpression of PPARγ1 leads to adipogenic hepatic steatosis.

MATERIALS AND METHODS

Mice and Treatment—Wild type (C57BL/6J) mice and PPARα−/−mice (22), 3 to 4 months of age and weighing 25–35 g, were used in this study. PPARα−/−mice were maintained on powdered chow with or without troglitazone (0.1% w/w) for 5 days prior to adenovirus injection and killed on day 2, 3, 4, 5, or 6 after injections while still on the same diet. For dose response of Ad/PPARγ1, mice were maintained on powdered chow, injected with different concentrations of virus, and killed 6 days after injection. For the induction of fatty liver, PPARα−/−mice were either fasted for 96 h (23) or fed a choline-deficient diet (Dyets, Bethlehem, PA) for 15 days (24, 25). All animal procedures used in this study were reviewed and preapproved by the Institutional Review Boards for Animal Research of the Northwestern University.

Primers for Endogenous Mouse PPARγ—To assess the level of expression of endogenous PPARγ1 under forced expression of Ad/PPARγ1, the following primers were used to distinguish the ectopic expression from endogenous. They are endogenous PPARγ1 sense: 5′-ctggagggcagccggaagag-3′; endogenous PPARγ2-sense: 5′-tgacccaagactgtgtggcc-3′; adenoviral PPARγ1-sense: 5′-cggagcttcatcatgaggtcttc-3′. All three PPARγ RT-PCRs were performed using the same antisense primer, 5′-tgggtggtggccagccggaagaag-3′. Primers for the internal control β-actin were sense: 5′-gtctgtcctagctggctggc-3′, antisense: 5′-gtctgagccactctggtggc-3′. PCR reactions were denatured at 94 °C for 2 min, cycled at 94 °C for 10 s, 60 °C for 30 s at 72 °C for 45 s, 30 cycles for PPARγ and 25 cycles for β-actin by using the GeneAmp PCR system 9700 (PE Applied Biosystems).

Adenoviral Gene Transfer—Construction of recombinant adenovirus containing the mouse PPARγ1 cDNA (Ad/mPPARγ1) was as follows. Mouse PPARγ1 cDNA (8) was cloned into pShuttle-CMV expression vector at ScoI site (Quantum Biotechnologies, Inc.). The linearized shuttle vector and AdEasy vector (Quantum Biotechnologies, Inc.) were then co-transformed into Escherichia coli strain BJ5183. Positive recombinant plasmid Ad/mPPARγ1 was selected. The Ad/mPPARγ1 virus was then generated as described previously (26). Adenoviral construct of Ad/LacZ was the generous gift of Dr W. El-Deiry (University of Pennsylvania, Philadelphia, PA) and has been described (27). Mice were intravenously injected (tail vein) in a volume of 200 μl with 1 × 1011 virus particles of Ad/LacZ or Ad/mPPARγ1 and killed 6 days later. Mice injected with PBS served as controls in some cases.

Morphology—Tissue fixed in 10% neutral buffered formalin was embedded in paraffin by using standard procedures. Sections (4–μm thick) were cut and stained with hematoxylin and eosin. For visualization of pathological changes, sections were counterstained with methyl green (24, 25). Tissue fixed in 10% neutral buffered formalin was also used for histological examination. Tissue fixed in 10% formalin was postfixed with 1% osmium tetroxide and embedded in low viscosity epoxy resin (26). Sections (1 μm thick) were stained with uranyl acetate and lead citrate and examined with a JEM-1230 electron microscope (JEOL, Tokyo, Japan). For ultrastructural analysis, tissue was fixed in 4% paraformaldehyde, dehydrated, and embedded in low viscosity epoxy resin (26). Sections (1 μm thick) were stained with uranyl acetate and lead citrate and examined with a JEM-1230 electron microscope (JEOL, Tokyo, Japan).

Northern and Immunoblot Procedures—Total RNA isolated from liver using Trizol reagent (Invitrogen) was glyoxylated, electrophoresed on 0.8% agarose gel, and then transferred to nylon membrane. These nylon membranes were then hybridized at 42 °C in 50% formamide hybridization solution using 32P-labeled cDNA probes. Equal loading was verified by the intensity of methylene blue-stained 18 S and 28 S RNA or by hybridizing the filters for glyceraldehyde-3-phosphate dehydrogenase. For immunoblotting, liver extracts were subjected to 7.5% or 10% SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted as previously described (26, 28, 29). The aP2 antibody was a generous gift from Dr. G. S. Hotamisligil (Harvard School of Public Health, Boston, MA).

RESULTS

Fatty Liver Resulting from PPARγ1 Overexpression—We have investigated the morphological changes in the liver of PPARα−/−mice injected with 1 × 1011 adenoviral-PPARγ1 particles intravenously. PPARγ1 overexpression in the liver of PPARα−/−mice caused extensive lipid accumulation in hepatocytes located in the peripheral and midzonal regions of liver lobules at 6 days (Fig. 1). The accumulation of fat in liver was grossly evident between 3 and 6 days after Ad/PPARγ1 injection. The degree and zonality of lipid accumulation in liver in mice overexpressing PPARγ1 appeared essentially similar whether or not they were on troglitazone, the PPARγ1 ligand (Fig. 1, B and C). Mice given troglitazone and injected with either PBS or with Ad/LacZ failed to reveal lipid accumulation in hepatocytes (Fig. 1, A and D). Oil red O staining of liver
sections obtained from PPARα−/− mice infected with Ad/mPPARγ1 confirmed hepatic lipid accumulation (Fig. 1E), whereas no appreciable lipid accumulation was evident in the liver of mice treated with Ad/LacZ (Fig. 1F). Immunohistochemical analysis revealed PPARγ nuclear staining in ~70% of hepatocytes with microvesicular steatosis between 4 and 6 days following Ad/mPPARγ1 injection into the PPARα−/− mice (Fig. 1G). No detectable immunostaining for PPARγ was noted in the livers of Ad/LacZ-injected mice (Fig. 1H). As expected, ~60–70% of hepatocytes stained positively for β-galactosidase after Ad/LacZ injection (Fig. 1D). Wild type (PPARα+/−) mice dosed with Ad/mPPARγ1 also exhibited hepatic lipid accumulation but not as severe as that seen in PPARα−/− livers with PPARγ overexpression (not illustrated), suggesting that the presence of PPARγ in the liver facilitates the up-regulation of fatty acid oxidation systems and reduces lipid accumulation (11, 23).

PPARγ1-induced Adipogenic Hepatic Steatosis

FIG. 2. Inducibility of PPARγ and PPARα target gene expression in PPARγ−/− mouse liver. A, Northern blot showing the expression levels of PPARγ, aP2, adipin, adiponectin, G6-P, straight chain fatty acyl-CoA oxidase, L-PBE, and peroxisomal 3-ketoacyl-CoA thioester genes after viral injection. PPARα−/− mice maintained on troglitazone were injected with 1 × 1011 Ad/mPPARγ1 viral particles and killed at 2, 3, 4, 5, and 6 days after infection. PPARα−/− mice injected with Ad/LacZ were killed 6 days after the injection. This blot includes samples from 2, 3, and 6-day post-treatment groups (2 animals). RNAs, 28 S and 18 S, are shown as a measure of loading control. B, Northern blot of C/EBPα, SREBP1, glucokinase, phosphoenolpyruvate carboxykinase, Glut-2, low density lipoprotein receptor, aP2, microsomal triglyceride transfer protein, CD36, Δ9d and other genes after viral injection. PPARα−/− mice (three in each group) maintained on normal diet were infected with 1 × 1011 Ad/mPPARγ1 or Ad/LacZ viral particles and killed 6 days after infection. Total RNAs from liver samples were hybridized with aforementioned probes. Glyceraldehyde-3-phosphate dehydrogenase is used as a loading control. C, immunoblot of PPARγ, aP2, and L-PBE genes. PPARα−/− mice (two in each group) maintained on troglitazone were infected with 1 × 1011 Ad/mPPARγ1 viral particles and killed 2, 3, 4, 5, and 6 days after infection. PPARα−/− mice injected with Ad/LacZ were killed 6 days after injection. Liver samples were immunoblotted for PPARγ, aP2, L-PBE, and catalase. The nonresponsive gene catalase was shown as loading control.

FIG. 3. Expression of aP2 in PPARγ-induced adipogenic hepatic steatosis but not in other forms of fatty liver. A, immuno blot analysis of aP2 expression in PPARγ−/− PPARα−/− wild type mouse liver. Mice were injected with PBS, Ad/LacZ, or Ad/PPARγ1 through tail vein with or without treatment with troglitazone and killed 6 days later. Liver samples from each group were immunoblotted for PPARγ and aP2. The nonresponsive gene catalase is used as loading control. B, expression of PPARγ and aP2 genes in fatty livers. Steatosis that developed in PPARγ−/− mouse liver after infection with Ad/PPARγ1 for 6 days (lanes 1 and 2), following fasting for 4 days (lanes 3 and 4), or fed a choline-deficient diet for 15 days (lanes 5 and 6). Representative liver samples were immunoblotted (two mice in each group) for PPARγ, aP2 (PPARγ-responsive gene), and catalase (nonresponsive gene). C, Northern blot analysis of RNA obtained from fatty livers that developed in PPARα−/− mice after infection with Ad/PPARγ1 (6 days), starvation for 4 days, or after 2 weeks of feeding a choline-deficient diet. Expression of adipogenesis-associated genes is confined to PPARγ-induced adipogenic hepatic steatosis and not with other forms of fatty liver.

FIG. 4. Histological evidence of hepatic steatosis in PPARα−/− mice. A, fatty liver following 4 days of starvation. B, fatty liver after 2 weeks of feeding a diet deficient in choline. C, hepatic steatosis in Ad/PPARγ1-injected mouse. Insets show high magnification of red areas. Note the presence of steatohepatitis in choline-deficient liver.
| GenBank Accession | Fold induction | Gene |
|-------------------|----------------|------|
| Adipogenesis      |                |      |
| X04673            | ~137.5         | Adipsin |
| M20497            | 66.5           | aP2 adipose fatty acid-binding protein (422) |
| U99915            | ~47.8          | Adiponectin |
| A1747654          | ~27.6          | Caveolin-1 |
| U10374            | 22.7           | Peroxisome proliferator activator receptor γ |
| A1846720          | ~13.0          | Voltage-gated potassium channel protein KV3 |
| M61737            | ~11.5          | Fat specific gene 27 |
| A1118905          | ~8.3           | SMAF1 (adipose differentially expressed gene) |
| AF064748          | ~6.0           | S3–12 gene |
| Lipid metabolism  |                |      |
| AJ239065          | ~57.1          | Keratinocyte lipid-binding protein |
| AB018421          | ~36.7          | Cytochrome P-450 4A10 |
| Y11638            | 29.9           | Cytochrome P-450 4A14 |
| U69543            | 13.2           | Hormone-sensitive lipase |
| AI846600          | 12.6           | Monoglyceroide lipase |
| AA797604          | ~11.5          | PIAF |
| AV238359          | 11.3           | Carnitine acetyltransferase |
| Z50024            | ~10.6          | Phosphatidylethanolamine transfer protein |
| AV090583          | ~8.5           | Farnesyl diphosphate synthetase |
| AF078752          | 8.1            | Diacylglycerol acyltransferase |
| L09192            | 7.4            | Pyruvate carboxylase |
| AB013874          | ~7.3           | Low density lipoprotein receptor-related protein 4 |
| M25944            | 7.2            | Carbonic anhydrase II (CAII) |
| AI539004          | 7.1            | Long chain fatty acid elongase |
| L23108            | ~6.8           | CD36 antigen |
| A1530403          | 6.2            | Peroxisomal 3-oxoacyl-CoA lyase A thiolase |
| AA883883          | 6.1            | Mitochondrial carrier; dicarboxylate transporter |
| Metabolism        |                |      |
| AJ647548          | ~27.1          | Cytochrome P-450 4B1 |
| AJ132098          | 20.3           | Vanin-1 |
| AV290060          | ~11.7          | Glycerocephosphate dehydrogenase |
| U469350           | 11.0           | Mus musculus CTP synthetase |
| AW122413          | ~10.1          | Metabolite transporter |
| AF007267          | ~9.8           | Phosphomannomutase Sec53p homolog |
| D64162            | 9.3            | Retinoic acid early transcript 7 |
| A0153547          | ~8.7           | Serine protease inhibitor 14 |
| L31763            | 8.6            | Urdin kinase |
| U51014            | 7.1            | Peptidase 4 |
| U17282            | 6.3            | Gastric H+–K+–ATPase α subunit |
| Y12577            | ~6.1           | ADP-ribose synthesis-like 4 |
| Apoptosis, signaling pathway and cell cycle | | |
| AF041376          | ~101.0         | Cell death activator CIDE-A (Cide-a) |
| M74227            | ~32.6          | Cyclophilin C (cyc C) |
| AW121234          | ~20.1          | G-protein modifier RAB-GTPase activation protein-related |
| U22398            | 19.0           | Cyclooxygenase-dependent kinase inhibitor 1C (P57) |
| X16995            | ~14.8          | Nur77 |
| AV054196          | ~11.4          | Sin3-associated polypeptide 18 |
| X119092           | ~7.8           | MAP kinase-interacting serine/threonine kinase 2 |
| Cytoskeleton      |                |      |
| AF057156          | ~22.6          | Mus musculus small proline-rich protein 1A (Sprr1A) |
| X04663            | ~10.6          | β-Tubulin (isotype Mbeta 5) |
| M31058            | ~8.3           | Muscle-skeletal muscle β-tropomyosin |
| AF020790          | ~7.1           | Keratin complex 1, acidic, gene 24 |
| U43298            | ~6.4           | Laminin, β 3 |
| Miscellaneous     |                |      |
| U41341            | ~26.3          | S100 calcium-binding protein A11 (calizzarin) |
| AI839175          | ~17.5          | Serum deprivation response |
| M60348            | ~16.9          | Multidrug resistance protein (MDR1) |
| AA793009          | ~16.1          | Tesfis expressed gene 19 |
| X63782            | 12.3           | Lymphocyte antigen 6 complex, locus D |
| V06566            | ~9.5           | Whey acidic protein |
| AA790008          | 9.3            | Reduced expression 3 |
| D64162            | 9.3            | Retinoic acid early transcript gamma |
| AA015547          | ~8.7           | Serine protease inhibitor 14 |
| U47323            | ~8.4           | Stromal interaction molecule 1 |
| J04634            | ~6.1           | Lymphocyte antigen 64 |
| Uncharacterized genes |              |      |
| AI850713          | ~17.2          | Similar to hypothetical protein from clone 24796 |
| AI834895          | 10.7           | RIKEN cDNA 2310021M12 gene |
| AW049551          | ~9.0           | Similar to hypothetical protein from clone 643 |
| AI848416          | 8.7            | Expressed sequence AW298908 |
| AW045632          | ~8.1           | RIKEN cDNA 1110001J03 gene |
| AI835260          | 8.0            | RIKEN cDNA 5830482G23 gene |
| AI840078          | 7.4            | Hypothetical protein MGC37904 |
| AW121217          | 6.8            | RIKEN cDNA 0610039C21 gene |
| AW120925          | ~6.5           | RIKEN cDNA 2610012022 gene |
| AI839718          | ~6.3           | RIKEN cDNA 6530401D17 gene |
| AI842715          | 6.2            | RIKEN cDNA 2310001A20 gene |
| AW048729          | 6.0            | Highly similar to sodium-dependent multivitamin transporter |
expression of PPARγ2 is restricted mainly to adipocytes and is the key regulator of adipogenesis, although forced expression of PPARγ1 isoform can also induce adipogenesis in fibroblasts (2, 14). Since we noted a striking degree of lipid accumulation in hepatocytes following PPARγ1 overexpression, it appeared necessary to investigate the adipogenic action of overexpressed PPARγ1 in liver (Fig. 2). Northern analysis of RNA isolated from PPARγ1−/− mouse liver revealed dramatic induction of mRNAs for fat differentiation markers aP2, adipin and adiponectin (adipoQ/acrp30) (see Refs. 31, 32) in PPARγ1 overexpressing livers but not after Ad/LacZ injection (Fig. 2, A and B). Since these mice are PPARγ−/−, it is reasonable to conclude that PPARγ plays no role in the observed induction of these adipogenic genes. The mRNA of the adipin gene reached peak level at 3 days postinjection, whereas aP2 mRNA level was maximally expressed at 6 days after Ad/PPARγ1 injection (Fig. 2A). The induction of adiponectin appeared somewhat delayed when compared with aP2 and adipin. Glucose-6-phosphatase (Glc-6-P) mRNA level in liver increased 2 days after Ad/PPARγ1 injection (Fig. 2A). We also determined the hepatic mRNA levels of several genes 6 days after Ad/PPARγ1 injection to assess the adipogenic and lipogenic profile. Noticeable increases in CD36, glucokinate, malic enzyme, low density lipoprotein receptor, microsomal triglyceride transfer protein, and Δ5d mRNA levels were detected at 6 days after Ad/PPARγ1 injection (Fig. 2B). We did not observe perceptible increases in the levels of C/EBPα, SREBP, glucokinate, phospho(eni)pyruvate carboxykinase, and Glut-2 mRNAs in liver (Fig. 2B). We also assessed the mRNA levels of PPARγ-regulated peroxiosomal fatty acid β-oxidation system genes and found modest increases in straight chain fatty acyl-CoA oxidase, L-PBE, and peroxiosomal 3-ketoacyl-CoA thiolase mRNAs (Fig. 2A), suggesting that PPARγ1 at very high levels can transcriptionally activate PPARγ target gene expression and regulate fatty acid β-oxidation in liver in the absence of PPARα.

Immunoblotting confirmed the induction of white adipose tissue marker protein aP2 in PPARγ1-overexpressing liver beginning at day 3 (Fig. 2C). This protein was not detected in normal liver or Ad/LacZ-infected mouse livers (Fig. 3C). Increase in PPARγ1 and in L-PBE protein levels were also seen in Ad/PPARγ1-expressing livers (Fig. 2C). As expected, no change in the amount of catalase protein, the peroxisomal marker enzyme, was discerned.

**TABLE II**

| GenBank accession | Fold reduction | Gene |
|-------------------|---------------|------|
| **Known genes**   |               |      |
| M64085            | ~19.3         | Mouse spi2 proteinase inhibitor (spi2/eb1) |
| X04283            | 17.7          | Cytochrome P2-450 |
| U43086            | 15.5          | Glucocorticoid-attenuated response gene 49 |
| J03858            | 14.4          | Glutathione S-transferase, alpha 2 (Yc2) |
| U43084            | 11            | Interferon-induced protein with tetratricopeptide repeats 1 |
| U15350            | ~9.8          | Histocompatibility 2, class II, locus Mb1 |
| J04596            | 9.7           | Chemokine (C-X-C motif) ligand 1 |
| X03505            | 9.3           | Serine protease inhibitor 2.4 |
| X08932            | 9.1           | Lipocortin-encoding 24p3 gene |
| X18162            | 8.8           | Flavin-containing monoxygenase 3 |
| U57147            | 8.6           | Interleukin-8-binding protein |
| M90551            | 8.6           | Intercellular adhesion molecule 5 |
| A0307970          | 8.6           | mGBP-2 protein |
| X53042            | ~8.6          | Asialglycoprotein receptor 2 |
| Z83815            | ~8.6          | Dystrophia myotonia kinase, B15 |
| U838325           | 8.5           | Cytokine inducible SH2-containing protein 7 |
| U430865           | ~8.4          | Interferon-induced protein with tetratricopeptide repeats 2 |
| AB019505          | 8.3           | Interleukin-18-binding protein |
| M55444            | 8.2           | Guanylate nucleotide binding protein 1 |
| AF080469          | 8             | Putative glycogen storage disease type 1b protein |
| U94829            | 7.9           | Retinoid abundant regulator of G-protein signaling RGS-1 |
| X56602            | 7.8           | Interferon-induced 15-KDa protein |
| Y12997            | 7.5           | Carboxylesterase |
| M32366            | 7.3           | Murine macrophage interferon inducible protein 10 (IP-10) |
| U35323            | 7.2           | H2-M alpha chain gene, H2-M beta 2 chain gene, H2-M β 1 chain gene, low molecular weight protein 2 (Lmp2q) gene |
| AW047476          | 6.8           | Guanylate nucleotide-binding protein 3 |
| AF047542          | 6.7           | Cytochrome P450 (Cyp2c37) |
| U65403            | ~6.7          | Serum amyloid A 4 |
| A123967           | ~6.7          | Immunoresponsive gene 1 |
| Y12657            | ~6.4          | Cytochrome P450, 26, retinoic acid A1 |
| M63630            | 6.1           | Interferon γ inducible protein, 47 kDa |
| Z38774            | 6             | α-2 antiplasmin |

**Uncharacterized genes**

| GenBank accession | Fold reduction | Gene |
|-------------------|---------------|------|
| AW120882          | ~14           | RIKEN cDNA 2310016E22 gene |
| AV1522044         | 10.1          | Mouse UniGene Cluster Mm.204646 |
| AW265885          | 9             | Mouse UniGene Cluster Mm.27805 |
| AA204579          | ~8            | Mouse UniGene Cluster Mm.27267 |
were down-regulated in Ad/PPAR overexpression, we found that 184 genes were up-regulated and 87 genes were down-regulated by a 4-fold change is used as the cut off for either up- or down-regulation. Affymatrix microarray chips containing 12,000 genes. When a target gene aP2, adipsin, and adiponectin and that PPAR

from either Ad/PPAR overexpression induced the adipogenic aP2 protein as well as hepatic adipogenesis (hepatic adiposis) from the typical non-alcoholic hepatic steatosis (30). We induced fatty liver in PPARα−/− mice (Fig. 4) by either fasting these mice for 96 h (23) or feeding them a diet deficient in choline for 15 days (24). Immunoblotting failed to reveal PPARα and aP2 protein in fatty livers induced by starvation or by a choline-deficient diet, but these two proteins appeared prominent in the fatty liver induced by PPARα overexpression (Fig. 3D). These observations clearly demonstrate that hepatic fatty change itself is not enough to induce either PPARα or its target gene aP2, adipsin, and adiponectin and that PPARα overexpression leads to a novel type of adipogenic hepatic steatosis designated hepatic adiposis to distinguish it from the common hepatic steatosis.

Gene Expression Profiling—To investigate whether PPARγ1 overexpression in liver indeed leads to adipogenic transdifferentiation of hepatocytes, we performed global transcriptional profiling using RNA isolated from liver 6 days after injection of Ad/PPARγ1 into PPARα−/− mice. Biotin-labeled RNA probes from either Ad/PPARγ1 liver or Ad/LacZ liver were hybridized to Affymatrix microarray chips containing 12,000 genes. When a 4-fold change is used as the cut off for either up- or down-regulation, we found that 184 genes were up-regulated and 87 were down-regulated in Ad/PPARγ1-overexpressing livers. Table I lists transcripts displaying a 6-fold or greater increase in expression induced to lipid metabolism indicating that the observed lipid accumulation in hepatocytes reflects transformation/transdifferentiation of hepatocytes toward adipocytes. Adipocyte marker genes adipin (33), aP2 (34, 35), adiponectin (36, 37), and cavelolin-1 (38, 39) were increased to −137, −66, −48, and −28-fold, respectively. E-FABP (40), FSP27 (41), CYP4A10 and CYP4A14 (25), hormone-sensitive lipase (42), monoclyceride lipase (43), and others involved in lipid metabolism were induced >10-fold in PPARγ1-overexpressing livers. Three apoptosis-associated genes, namely cell death-inducing DNA fragmentation factor (CIDE) (44), cyclophilin C (45), and nur77 (46, 47) were increased to −101, −33, and −15-fold, respectively, suggesting that induction of the adipogenic differentiation program in liver augments hepatocyte apoptosis. Northern analysis confirmed marked increases in the levels of cavelolin-1, CIDE-A, and nur77 mRNAs in liver beginning about 3 days after Ad/PPARγ1 injection (Fig. 5). Previously, we found up-regulation of CIDE in the liver following treatment with WY-14,643, a PPARα ligand (43). However, since CIDE was not up-regulated in mice lacking straight chain fatty acyl-CoA oxidase, which exhibit spontaneous PPARα activation, we concluded that induction of CIDE may not be dependent upon PPARα (43). Further studies are needed to ascertain the mechanisms and implications of CIDE, cyclophilin C, and nur77 up-regulation following overexpression of PPARγ1 in liver. Among the genes up-regulated between 5- and 10-fold are farneyl diphosphate synthetase (−9-fold), diacylglycerol acyltransferase (−8-fold), pyruvate carboxylase (−7-fold), long chain fatty acyl elongase (−7-fold), CD36 (−7-fold), adiponutrin-like protein (−7-fold), acetyl-CoA acyltransferase (−6-fold), dicarboxylate transporter (−6-fold), and S3–12 gene (−6-fold), among others (Table I). A majority of these genes participate in lipid metabolism.

Table II lists the genes that were down-regulated 6-fold or higher in the liver with PPARγ1 overexpression. These include genes encoding for interferon-induced protein with tetratricopeptide repeats 1 and 2, interferon-induced 15-kDa protein, interferon-γ-induced 47-kDa protein, and asialoglycoprotein receptor among others.

Endogenous PPARγ Expression—We have ascertained that forced expression of PPARγ1 does not up-regulate endogenous PPARγ (Fig. 6). PPARγ2 sense primer was designed to include the N-terminal region of PPARγ2 to distinguish it from endogenous PPARγ1. For ectopic PPARγ1 the sense primer was from the cytomegalovirus promoter. The RT-PCR data show no increase in endogenous PPARγ1 or PPARγ2 mRNA in the liver 6 days after Ad/PPARγ1 injection.

DISCUSSION

The nuclear receptor PPARγ is essential for adipogenesis and lipid storage (1–4). PPARγ is present in two isoforms, PPARγ1and PPARγ2, generated by alternate promoter usage and splicing (9). PPARγ2 isoform, which is expressed exclusively in adipocytes, plays a pivotal role in adipocyte differentiation and adipocyte-specific gene expression, but there is some controversy as to whether PPARγ1 isoform can initiate and sustain adipogenic gene expression (2, 14, 48). Tontonoz et al. (2) reported that both PPARγ1and PPARγ2 could stimulate adipogenesis when introduced into fibroblasts, whereas Ren et al. (48), using engineered zinc finger repressors to inhibit the expression of PPARγ isoforms, have concluded that PPARγ1 overexpression exerted no adipogenic effect. Recently, Mueller et al. (14) using PPARγ null fibroblasts demonstrated convincingly that both PPARγ1and PPARγ2 isoforms have intrinsic ability to induce robust adipogenesis. While most of the work on PPARγ-induced adipogenesis involved the use of fibroblast cell lines, the key question as to whether PPARγ1 isoform, which is the only isoform expressed in liver and many other tissues, can stimulate fat cell differentiation or transformation of hepatocytes into adipocytes. In this study, we have shown that overexpression of PPARγ1 in mouse liver induced adipocyte-specific gene expression as well as microvesicular steatosis in this organ. Furthermore, we have demonstrated that hepatic steatosis resulting from PPARγ1 overexpression is associated with adipogenic gene expression, whereas hepatic steatosis induced by starvation or developing after feeding a choline-deficient diet failed to stimulate adipogenic gene expression. Accordingly, we propose that PPARγ overexpression leads to the development of a novel form of hepatic steatosis, which is designated adipogenic hepatic steatosis or simply “hepatic adiposis” to distinguish this entity from the common forms of hepatic steatosis.
Our results showed the expression of adipocyte-specific genes in liver upon overexpression of PPARγ1 whether or not these mice were treated with troglitazone. This may be due to the availability of putative endogenous ligands in liver generated as part of the normal lipid metabolism (49, 50). Because PPARγ1 is endogenously expressed at a low level in mouse liver it is unlikely that this receptor exerts any adipogenic effect under normal physiological conditions. Furthermore, the relative abundance of PPARα in normal liver serves as a key regulator of fatty acid catabolism thereby minimizing the need for adipogenesis in liver to store lipids (11, 30). In that sense functionally active PPARαs and fatty acid oxidation systems keep the PPARγ1 in check (30). Our results clearly establish that the overexpression of PPARγ1 isoform in liver in PPARα null background triggers the expression of adipogenesis-related genes and fatty change in liver. This process essentially represents conversion of hepatocytes into cells with active adipogenic gene expression profile, and in that regard this represents a transformation or transdifferentiation of hepatocyte into adipocytes, i.e., the conversion of fat-burning hepatocytes into fat storage cells (31, 32, 51). It is important to note that the expression of adipocyte-specific genes and microvesicular steatosis observed in liver is not associated with overexpression of the endogenous PPARγ gene. RT-PCR analysis showed no induction of endogenous PPARγ1 or PPARγ2 isoforms (Fig. 6). Thus all the changes observed in gene expression patterns and morphological changes are the result of the exogenous expression of adenovirally driven PPARγ1 overexpression. These results suggest that maintenance of low levels of PPARγ in liver is crucial for preventing hepatocytes from encountering an adipogenesis fate. The maintenance of marginal PPARγ1 gene expression in liver may be achieved by unknown mechanisms designed to prevent positive feedback between C/EBPα and PPARγ because the downstream adipogenic events could manifest successfully in the presence of abundant PPARγ protein (4).

We performed Northern analysis and global transcriptional profiling to define the pattern of genes expressed in liver as a result of PPARγ1 overexpression. Our studies indicate that overexpression of PPARγ1 isoform in liver results in the up-regulation of many genes known to be up-regulated during adipocyte differentiation of 3T3-L1 fibroblasts (31, 32, 52–54). Adipogenesis genes up-regulated in liver include PPARγ target genes adipin, aP2, adiponectin, caveolin, fat-specific protein 27, and others (Table I). Caveolae and caveolin-1 protein expression are most abundant in adipocytes (39, 55). The overexpression of caveolin-1 mRNA in liver expressing PPARγ is further indication of adipogenic transformation of hepatocytes in that caveolin-1 appears to participate in facilitating the conversion of triglycerides in lipoprotein form to triglycerides in lipid droplet storage from (39). Caveolin-1 appears functionally necessary to maintain lipid droplet integrity, and the absence of this protein in caveolin-1-deficient mice leads to abnormalities in adipocyte function (39). Increase in caveolin-1 gene expression in PPARγ1-overexpressing livers is further indication of attaining an adipocyte phenotype. In addition, numerous adipocyte-enriched genes involved in lipogenesis and lipid metabolism, in particular E-FABP (keratinocyte lipid binding protein) (40), cypr4a10, cypr4a14 (25), fasting-induced adipose factor (angiopterin-like-4) (56, 57), CD36 (58), glycerophosphate dehydrogenase (52), and hormone-sensitive lipase (42), were markedly up-regulated in liver following PPARγ1 overexpression. Thus, our in vivo observations clearly establish the adipogenic conversion of liver when PPARγ1 is overexpressed in this organ.

In this study, we noted marked up-regulation of three genes that have recently been shown to participate in apoposis. These include CIDE-A (44), cyclophilin C (45), and nur77 (46, 47). CIDEs belong to a novel family of recently identified cell death-inducing proteins that induce apoptosis when overexpressed (59). CIDE is not normally expressed in liver, but increased levels of CIDE mRNA have been noted in livers of mice that were treated with Wy-14,643, a PPARα ligand (49). However, the CIDE expression was not dependent upon PPARα (43). The functional implications of up-regulation of three apoptotic genes in liver with adipogenic hepatic steatosis suggests that the acquisition of adipogenic phenotype places hepatocytes at greater risk for apoptosis. Additional studies are needed to ascertain if the CIDE, cyclophilin C, and nur77 genes are PPARγ targets for regulation. Studies are also needed to ascertain if DNA synthesis is a prerequisite for the adipogenic conversion of hepatocytes.

It is of interest that this new form of hepatic adiposis or adipogenic hepatic steatosis resulting from PPARγ1 overexpression may have potential clinical implications. Individuals with relatively low hepatic levels of PPARα and hyperactive PPARγ1 resulting from single nucleotide polymorphism or by some other mechanism could develop adipogenic hepatic steatosis similar to that described in this report. It is also worth noting that ob/ob mice exhibit increased levels of PPARγ mRNA in their livers (60). Increased expression levels of aP2 and CD36 mRNA were seen in these livers when these mice were given PPARγ ligand troglitazone (61). Thus, it is important to consider PPARγ1 overexpression or hyperactivity as a possible molecular mechanisms responsible for a special type of non-alcoholic hepatic steatosis, designated as hepatic adiposis or adipogenic hepatic steatosis.

Finally, we have identified a number of novel genes as modestly up-regulated in PPARγ1-overexpressing liver, which remain to be characterized (Table I). This includes a gene we designated as promethin (AY167031), which is induced in liver by PPARγ1 overexpression. Additional studies are needed to further characterize these and other genes to gain appreciation of the role of PPARγ in liver in relation to glucose homeostasis, energy utilization, and insulin resistance (62).

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Adipocyte-specific Gene Expression and Adipogenic Steatosis in the Mouse Liver Due to Peroxisome Proliferator-activated Receptor γ1 (PPARγ1) Overexpression

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